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SUBSTITUTE CHEMICAL PROGRAM - THE FIRST YEAR OF PROGRESS

PROCEEDINGS OF A SYMPOSIUM

Volume IV

Chemical Methods Workshop

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SUBSTITUTE CHEMICAL PROGRAM - THE FIRST YEAR OF PROGRESS

PROCEEDINGS OF A SYMPOSIUM

Volume IV

Chemical Methods Workshop

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SHERATON-FREDERICKSBURG MOTOR INN
FREDERICKSBURG, VIRGINIA

JULY 30 - AUGUST 1, 1975

The Proceedings of the SUBSTITUTE CHEMICAL PROGRAM -- THE FIRST YEAR OF PROGRESS are published in four volumes. Volume I contains speeches and discussion from the Plenary Session, the agenda, and lists of participants and speakers. Volumes II, III, and IV cover the Toxicological Methods and Genetic Effects Workshop, the Ecosystems/Modeling Workshop, and the Chemical Methods Workshop, respectively.

TABLE OF CONTENTS

Page

Wednesday, July 30, 1975

MULTIRESIDUE METHODOLOGY
Dr. H. Anson Moye 1

IDENTIFICATION OF IMPURITIES IN TECHNICAL-GRADE PESTICIDES
Dr. J. S. Warner 31

SENSORY CHEMICAL PESTICIDE WARNING SYSTEM
Dr. Donald E. Johnson 67

Thursday, July 31, 1975

ANALYSIS OF PESTICIDES AND PESTICIDE METABOLITES BY HYPERFINE LABELING
Dr. Barry Commoner 99

MASS SPECTROMETRY METHODS DEVELOPMENT
Dr. Edward O. Oswald 117

AUTOMATED CLEANUP AND SPECIFIC DETECTOR SYSTEM FOR PESTICIDE RESIDUE ANALYSIS
Kenneth R. Hill 127

MULTIRESIDUE METHODOLOGY
Dr. Robert Moseman 141

PESTICIDES IN AMBIENT AIR
Dr. Robert Lewis 147

IN SITU METHOD FOR ORGANOPHOSPHATE INSECTICIDES
Dr. William T. Colwell 159

TOXIC POTENTIATORS AS BY-PRODUCTS OF ORGANOPHOSPHORUS INSECTICIDES
Dr. R. Fukuto 165

RESEARCH PROGRAMS OF THE CHEMISTRY BRANCH
Dr. Edward O. Oswald 173

SPECIAL INTEREST TOPICS AND GENERAL DISCUSSION 185

MICROELECTROLYTIC CONDUCTIVITY DETECTOR
Jack B. Dixon 205

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MULTIRESIDUE METHODOLOGY

Dr. H. Anson Moye*

I feel free now to at least present my list of what were last year the alternative chemicals or substitute chemicals. Our contract really was composed of two parts, the first of which involved gas chromatography methodology, the second of which involved liquid chromatography or high-speed liquid chromatography, if you will, methodology. The idea in the gas chromatography portion was to flesh out the available data on extraction efficiencies and gas chromatographic characteristics of these compounds.

We knew from the literature and past experience that some of them had no hope of being gas chromatographed. Some could be gas chromatographed with chemical derivatization. So we included a short study on two chemical derivatives for those compounds that seemed appropriate.

We were to take these compounds -- I will list them for you shortly -- and try gas chromatography. We had at our disposal a Microtech or Tracor, if you will, MT 220 equipped with dual electron capture detectors and the microelectrolytic conductivity detector that Mr. Dixon just described, a Varian 1200 with the Tracor flame photometric detector. So we had the capability of detecting essentially every element responsive to any element-specific detector of which you might conceive.

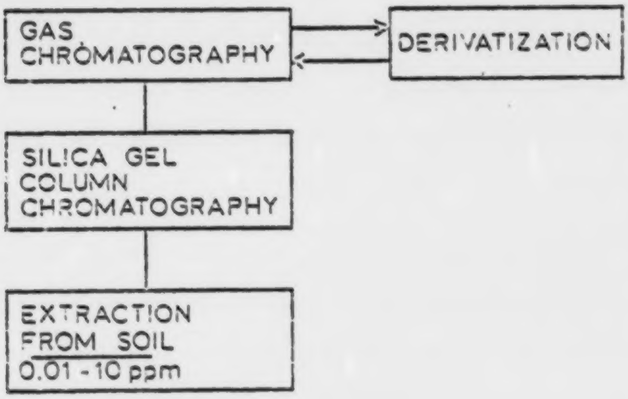
We were to attempt direct gas chromatography on these compounds. For those that could not be gas chromatographed we were to attempt to derivatize with two reagents which I will shortly describe (Figure 1). If we were successful in obtaining gas chromatographic responses, either of the intact pesticide or the derivatized pesticide, we were to apply a silica gel column cleanup which had been devised by

---

*Food Science Department, University of Florida, Gainesville, Florida

Drs. Shafik and Sherma, EPA, which had in previous work appeared to be somewhat superior to the Florisil cleanup that had been so long favored by the Food and Drug Administration and subsequently by EPA.

Figure 1



If we could get these materials through the silica gel column, we were then to go back, apply them to soils at levels of 0, 0.01, 0.1, 1.0 and 10 parts per million, perform extractions, do the column chromatography and then the derivatization when necessary, and then the gas chromatography.

In Figure 2 we broke our list of 33 compounds into three classes, mainly for bookkeeping mechanism whereby we could apply the element-specific detectors. We had a number of chlorine-containing compounds that were somewhat ambiguous such as trifluralin, which is not a chlorine-containing compound, but we didn't know where else to put it.

The phosphorus-containing compounds we examined were azinphos-ethyl, azinphos-methyl, Azodrin, demeton, malathion, methyl parathion, parathion, and phorate. The nitrogen-containing compounds were amitrole, atrazine, Avadex, benomyl, bromacil, Bux, CIPC, diquat, dinitro-orthocresol (DNOC), ferbam, Furadan, IPC, monuron, simazine, Temik or aldicarb, and Zectran. As you see from the list,

Figure 2

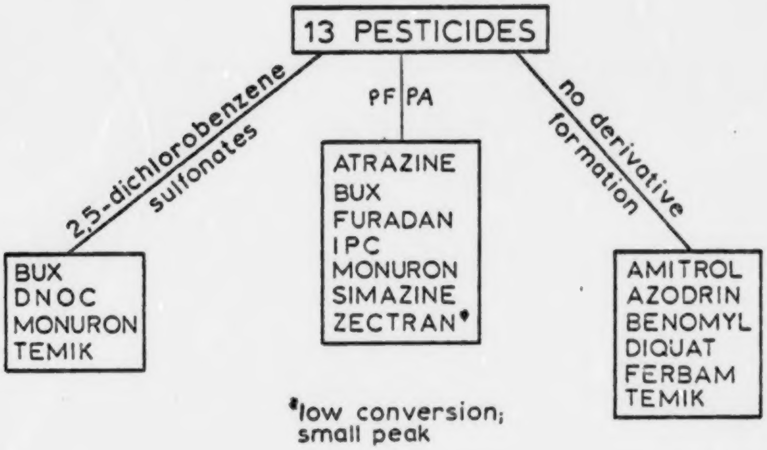
- 33 Pesticides
  - Chlorine-Containing
    - Captan
    - Chlorobenzilate
    - Difolatan
    - Endosulfan
    - Folpet
    - Methoxychlor
    - PCNB
    - Perthane
    - Trifluralin
  - Phosphorus-Containing
    - Azinophos Ethyl
    - Azinophos Methyl
    - Azodrin
    - Demeton
    - Malathion
    - Methyl Parathion
    - Parathion
    - Phorate
  - Nitrogen-Containing
    - Amitrole*
    - Atrazine*
    - Avadex
    - Benomyl*
    - Bromacil
    - Bux*
    - CIPC
    - Diquat*
    - DNOC*
    - Ferbam*
    - Furadan*
    - IPC*
    - Monuron*
    - Simazine*
    - Temik*
    - Zectran

---

*13 pesticides which could not be satisfactorily gas chromatographed intact.

those starred were the ones that we could not gas chromatograph intact. All the rest of them we could.

Figure 3


13 PESTICIDES
2,5-dichlorobenzene sulfonates
PFPA
no derivative formation
BUX
DNOC
MONURON
TEMIK
ATRAZINE
BUX
FURADAN
IPC
MONURON
SIMAZINE
ZECTRAN*
AMITROL
AZODRIN
BENOMYL
DIQUAT
FERBAM
TEMIK
*low conversion; small peak

On these 13 compounds we attempted two derivatization procedures. One is a Schotten-Baumann type reaction of 2,5-dichlorobenzenesulfonyl chloride which will react, we have shown, with essentially any N-methyl carbamate, although there are exceptions. The other reaction was a pentafluoropropionic anhydride derivatization that was also developed by Dr. Shafik, which had been shown to be applicable to any hydrogen-replaceable compound, such as N-methyl carbamate.

Of these 13 pesticides that were not gas chromatographable (Figure 3), there were essentially six that we could not gas chromatograph under any circumstances, with the exception of Temik or aldicarb. Temik did give a response with the 2,5-diachlorobenzene sulfonyl chloride; however, it formed in the methylamine mode, making it nonspecific. In other words, you could not separate it from materials like the methomyl.

We worked with four gas chromatographic columns, two of which were really holdovers or old-fashioned FDA columns, one a 10 percent DC-200 and one a 10 percent QF-1 (Figure 4). As you see, the 10 percent DC-200 appears to be far superior in its separatory power for 8 of the chlorinated compounds.

There are some ambiguities there. Endosulfan and perthane cannot be separated; that is, the high boiling isomer of endosulfan cannot be separated from perthane. QF-1 looks almost hopeless; everything kind of overlaps.

There were two mixed-phase columns which were examined (Figure 5). These were mixed-phase column packings that had been worked out by EPA and had been used in their community studies program, and, as you see, they do not do a much better job; however, the 4 percent SE-30, 6 percent OV-210 would be passable, I suppose. It had less retention than the DC-200 for everything essentially, but the efficiency was high enough that some reasonable separations could be obtained. The 1.5 percent OV-17, 1.95 percent OV-210 was not very good, to say the least.

Once we established the gas chromatographability of these materials, we tested them on the silica gel absorption column. Let me back up just a minute here. The four columns we tested had the theoretical plates calculated for a 6-foot long by 3 millimeter I.D. glass column. The DC-200, calculated on aldrin at 200° C, was only 1,700 theoretical plates (Table 1). So we really were not all that good in our column packing, but it was significantly superior to any of the three we packed in terms of efficiency.

The compounds that we could get gas chromatographed were applied to the activity number three silica gel column. This was a column 2.5 cm on the inside diameter, packed with 16 grams of Woelm activity three silica gel. This is a silica gel that is very closely controlled in its activity. It is supplied as activity three as a 15 percent moisture packing. It performed, I think, a remarkable

Figure 4. Chromatograms of an 8-compound chlorinated pesticide mixture. Column operating parameters are shown in Table 2.

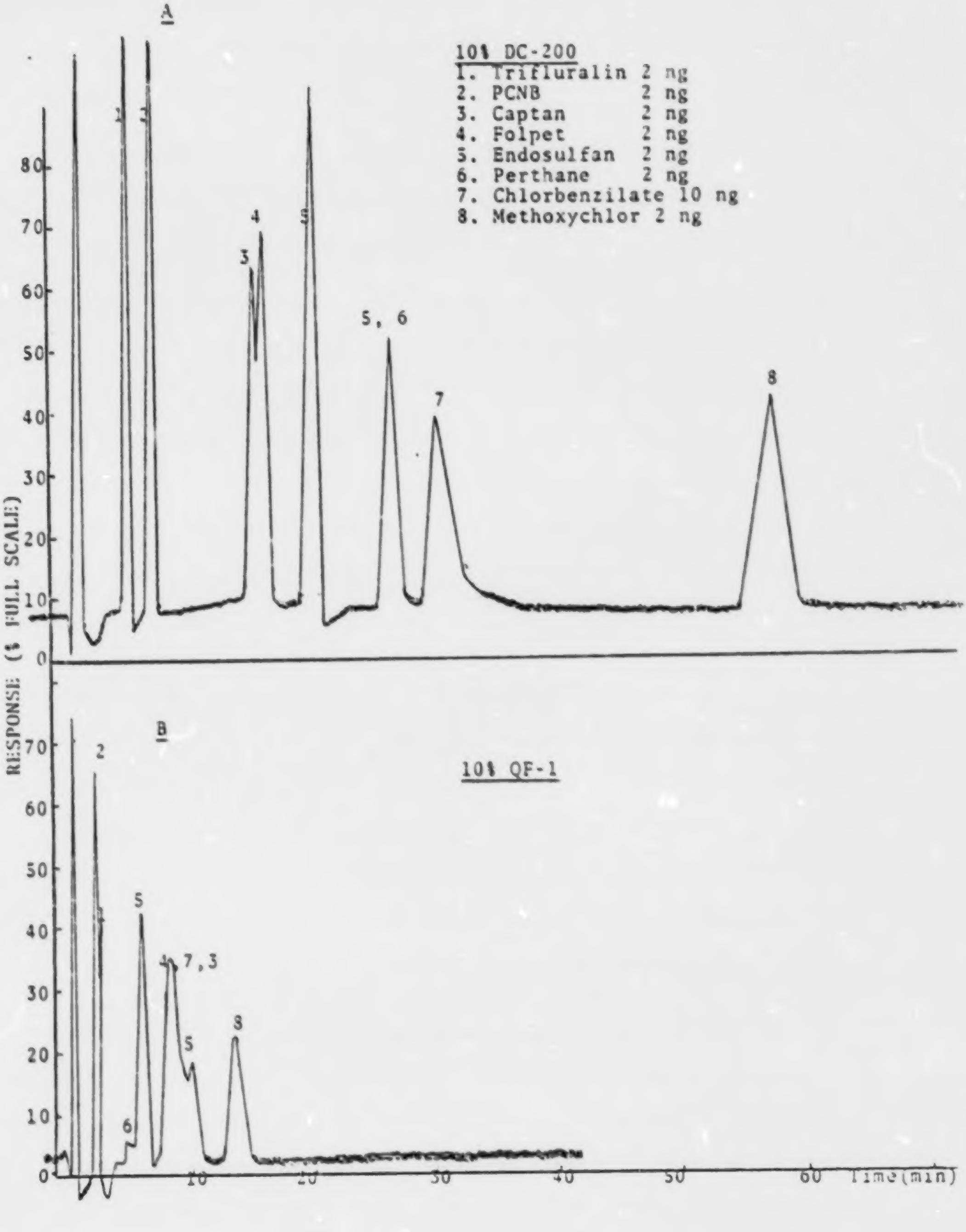


Figure 5. Chromatograms of an 8-compound pesticide mixture. Column operating parameters are shown in Table 3.

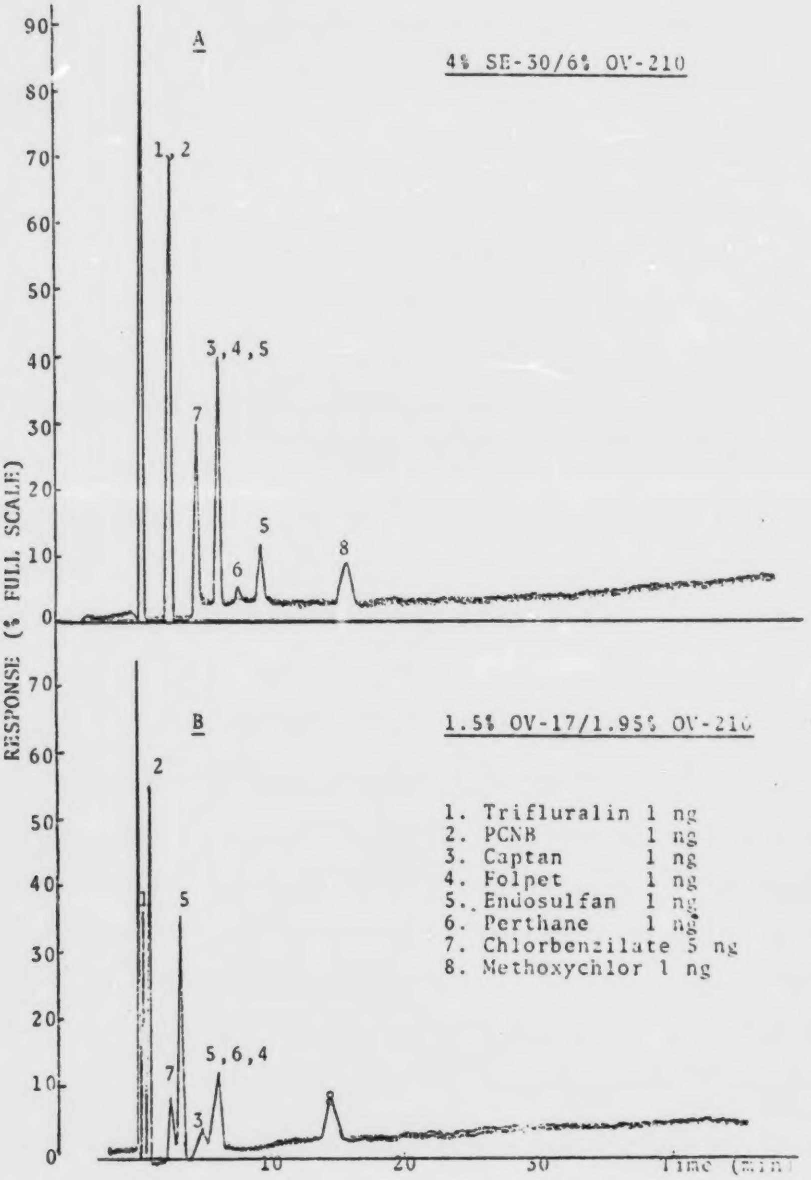


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Table 1: Computed Efficiencies of Columns
(tritium foil EC detector, Packard GLC).

Column	Efficiency(1) (Theor. Plates)
10% DC-200	1700 (2)
10% QF-1	676
4% SE-30/6% OV-210	1156
1.5% OV-17/1.95% OV-210	256

(1) Efficiency computed from total retention and base width of aldrin peak.
(2) Value represents superior efficiency.

fractionation of these materials. Table 2 shows the nitrogen-containing compounds and the halogen-containing compounds that we could gas chromatograph. If you look closely, you will see that there is essentially no holdover from one fraction to the other. This is quite remarkable, and I don't believe, to my knowledge, any other adsorption cleanup column can say this.

We found very, very little holdover. There are five fractions. The first fraction is eluted with 50 milliliters of hexane. This gives pentachloronitrobenzene. The second fraction is eluted with 60 percent benzene hexane. This gives the four that you see. The third fraction is 5 percent acetonitrile-benzene. This gives the seven that you see. The fourth fraction, 10 percent acetonitrile-benzene, and here we get the bulk of the carbamate or nitrogen-containing materials. Fraction five at 20 percent acetonitrile-benzene gets bromacil and monuron.

For the organophosphates listed in Table 3 once again a fractionation is observed. For the hexane fraction there is no organophosphate eluted. The 60 percent benzene hexane gives phorate, methyl parathion, parathion, and Azodrin; 5 percent acetonitrile-benzene, malathion, azinphos-methyl, azinphos-ethyl; and 10 percent

Table 2: Outline of Halogenated and Nitrogen-Containing Pesticide Standard (1 mg each) Eluted from Silica Gel Column

16 g silica gel, 14 g Na_2SO_4

Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V
Hexane	60% Benzene/ Hexane	5% Acetonitrile/ Benzene	10% Acetonitrile/ Benzene	20% Acetonitrile/ Benzene
PCNB(94%)	Trifluralin (105%) Perthane (97%) Methoxychlor(92%) Endosulfan (90%)	Captan (102%) Folpet (98.5%) Chlorobenzilate (91%) CIPC (90%) Difolatan (100%) DNOC (90%) Bux, IPC, Zectran	Atrazine (100%) Simazine (100%) Furadan (90%) Temik	Bromacil (98%) Monuron (75%)

Note: Percent recoveries are presented after each pesticide.

Table 3: Outline of Organophosphate Pesticides Recovered from Silt Loam Soil and Eluted from Silica Gel Column

16 g silica gel, 14 g Na_2SO_4

Fraction I	Fraction II	Fraction III	Fraction IV
Hexane	60% Benzene/Hexane	5% Acetonitrile/ Benzene	10% Acetonitrile/ Benzene
No pesticide	phorate methyl parathion parathion Azodrin	malathion azinphos-methyl azinphos-ethyl	demeton

acetonitrile-benzene, demeton. There is no fraction five; fraction five gives nothing. All of our organophosphates come off in the first four fractions.

The cleanup capability of this column, I think, is illustrated by Figure 6. This was a crude soil extract, and I might back up and say that we worked with three types of soil, and we performed all of our extractions at the various levels with all three types. We had a sandy soil, a sandy loam soil, and a silt loam soil. This is the sandy soil, which was taken from a cow pasture and had the highest organic content. It also gave the lowest percentage recovery for essentially all the compounds that we were able to recover satisfactorily, although not that much less.

You can see the crude extract on a DC-200 column. It is quite bumpy. There is no room to analyze anything at subpart per million levels with this crude extract. By putting it through the silica gel column, the hexane fraction shows a large proportion of the interferences. Pentachloronitrobenzene elutes in this fraction and then on the gas chromatogram, and the relative retention you see there.

In the 60 percent benzene hexane fraction (Figure 7) the materials elute as you see -- trifluralin, endosulfan, perthane, and methoxychlor. There is a little bit of clutter in the initial part of the chromatogram; however, trifluralin and endosulfan could be analyzed at the 0.01 part per million level. As in the next fraction, 5 percent acetonitrile-benzene, the elutions of CIPC, folpet, captan, chlorobenzilate, and difolatan are all at fairly clear places for the 0.01 ppm level.

The E fraction is still fairly clean in Figure 8. There is one major interference peak which elutes at about 5 minutes. Atrazine and simazine fall before that, just after one minor interference peak. The 20 percent acetonitrile fraction gives bromacil, which elutes in 5 minutes in almost a perfectly clean chromatogram.

Let me point out that the reaction involved in this is a pentafluoroproprionic anhydride derivatization. It is simply an acylation which takes place at room

Figure 6. Gas chromatograms of 50-mg equivalent crude soil extract and five silica gel column fractions from the total 100 g control methanol-benzene (1:1) Soxhlet extract of silt loam soil (retention times of pesticides were exhibited in appropriate chromatograms).

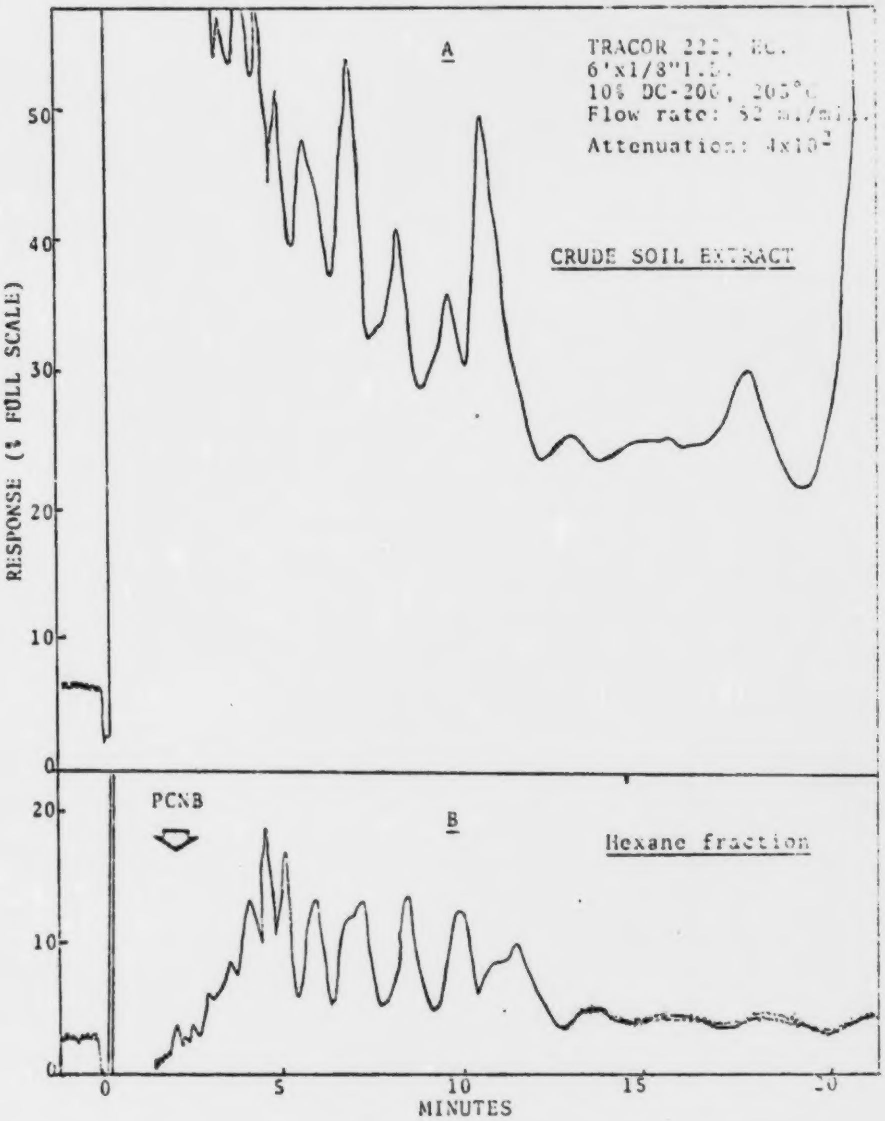

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Figure 7

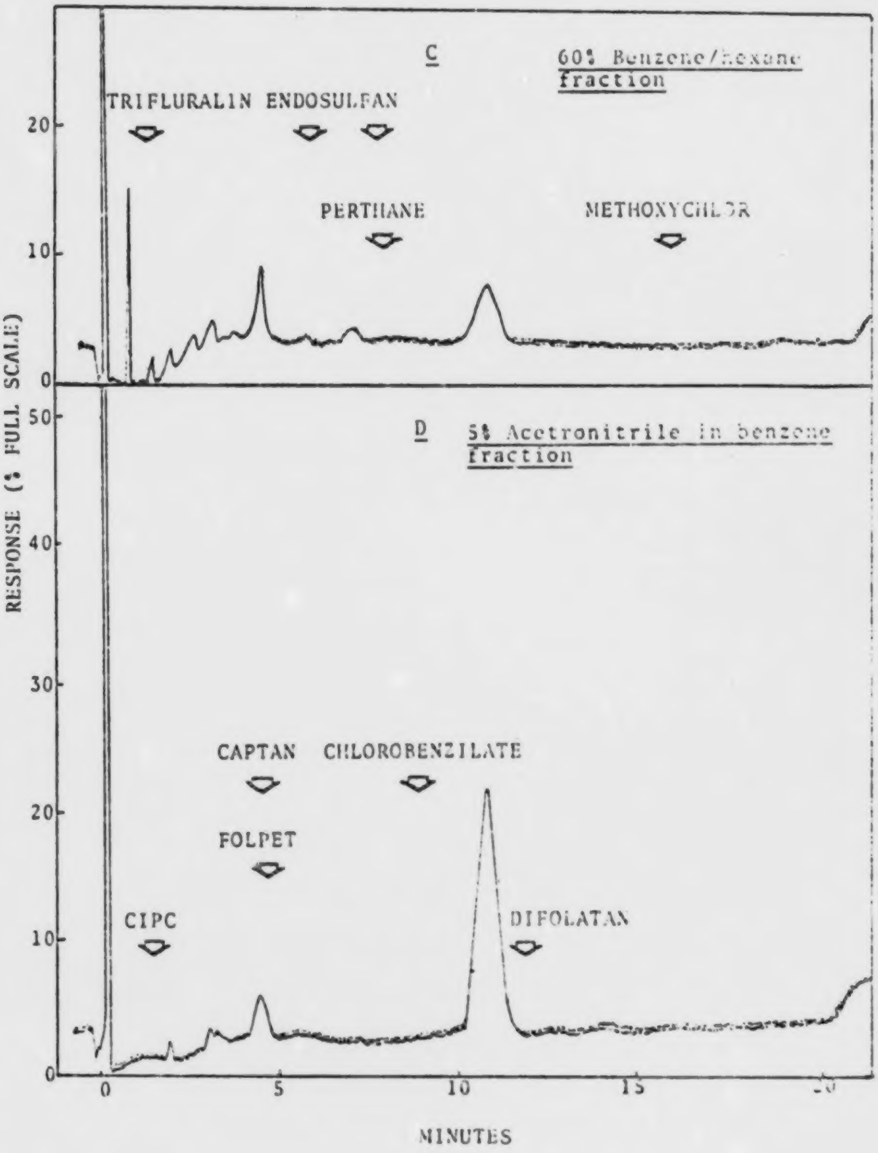
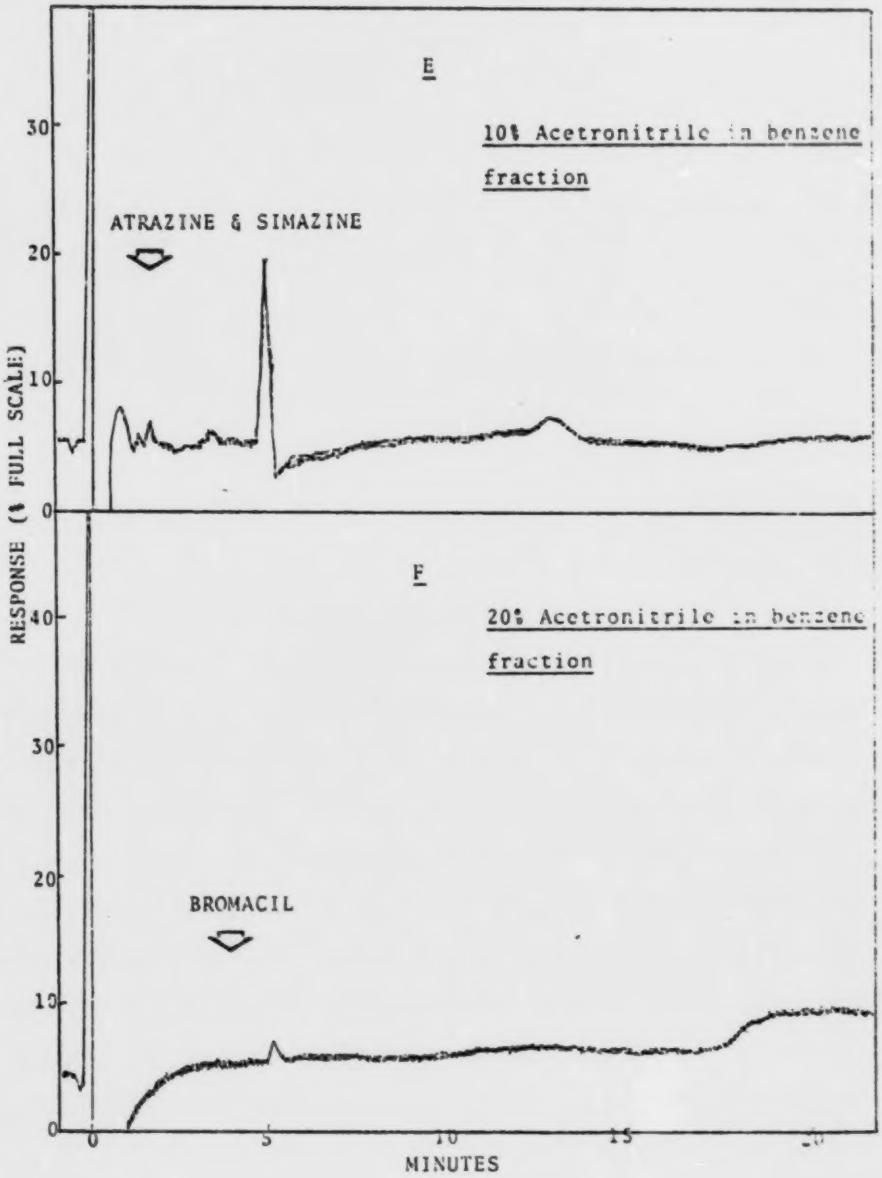
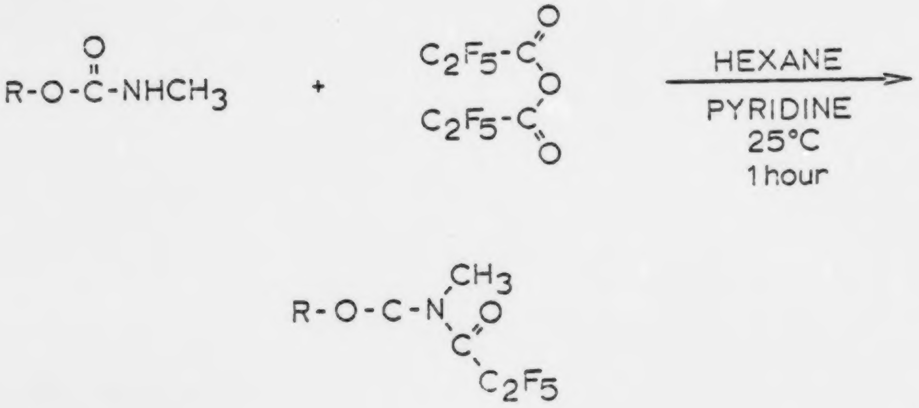

C
60% Benzene/hexane fraction
TRIFLURALIN
ENDOSULFAN
PERTHANE
METHOXYCHLOR
D
5% Acetronitrile in benzene fraction
CAPTAN
CHLOROBENZILATE
FOLPET
CIPC
DIFOLATAN
RESPONSE (% FULL SCALE)
0
10
20
30
40
50
0
5
10
15
20
MINUTES

Figure 8


E
10% Acetronitrile in benzene fraction
ATRAZINE & SIMAZINE
30
20
10
0
RESPONSE (% FULL SCALE)
F
20% Acetronitrile in benzene fraction
BROMACIL
40
30
20
10
0
0
5
10
15
20
MINUTES

temperature or for one hour in the presence of hexane and a small amount of pyridine (Figure 9).

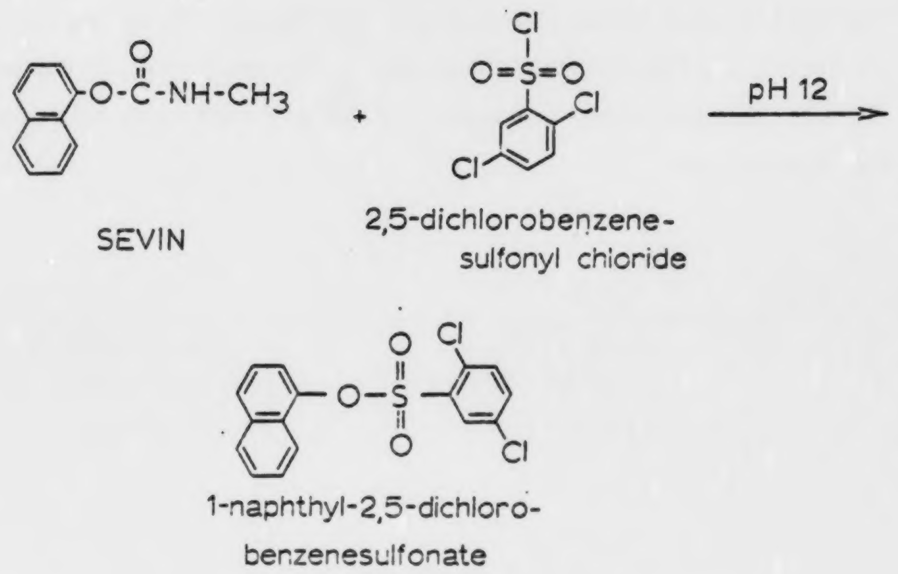
Figure 9



The reaction of 2,5-dichlorobenzenesulfonyl chloride is also an alkaline media base reaction (Figure 10). Here I don't show the conditions; however, it can be run in acetone or with a carbonate buffer. It can be run in hexane with a small amount of pyridine also, as is the PFPA derivative. Here I show it reacting with carbaryl, giving the sulfonate from the phenolic portion. One interesting thing about this reaction is, if you run this reaction with pyridine and hexane, you do not get a derivative for carbaryl. You get nothing. You don't get the sulfonamide, which would be produced by the reaction of 2,5-dichlorobenzenesulfonyl chloride and methylamine; however, when you do run this reaction with a pyridine medium in hexane, you can get methylamine derivatives from both aldicarb and methomyl, so the reaction seems to have a degree of specificity built into it, such that if you wanted to, you could screen for methomyl and aldicarb.

Figure 11 shows a reagent we have just begun to work with. It has recently been reported on in an article in JAOAC on ethylene thiourea, I believe. Anyway, it is

Figure 10



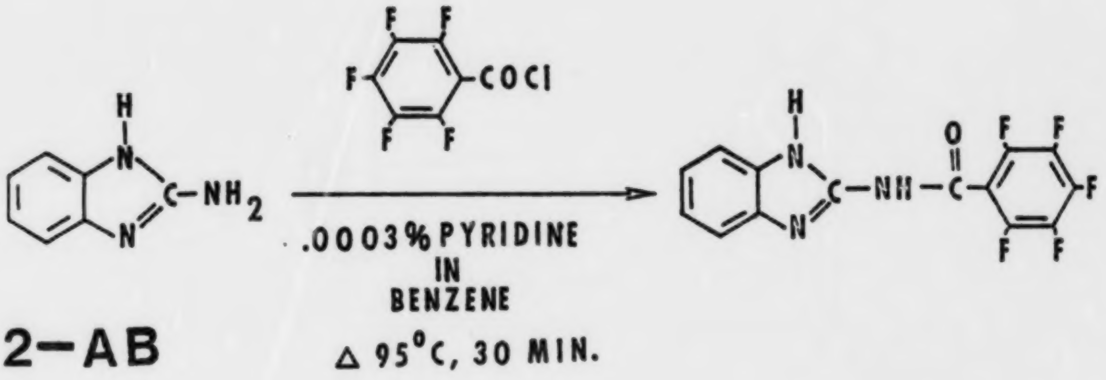
SEVIN

2,5-dichlorobenzenesulfonyl chloride

1-naphthyl-2,5-dichlorobenzenesulfonate

pentaflurobenzoyl chloride. It can be made to react quite quickly with 2-aminobenzimidazole, a metabolite of benomyl, and also it reacts with MBC, a metabolite of benomyl, and benomyl itself to give the exact same product.

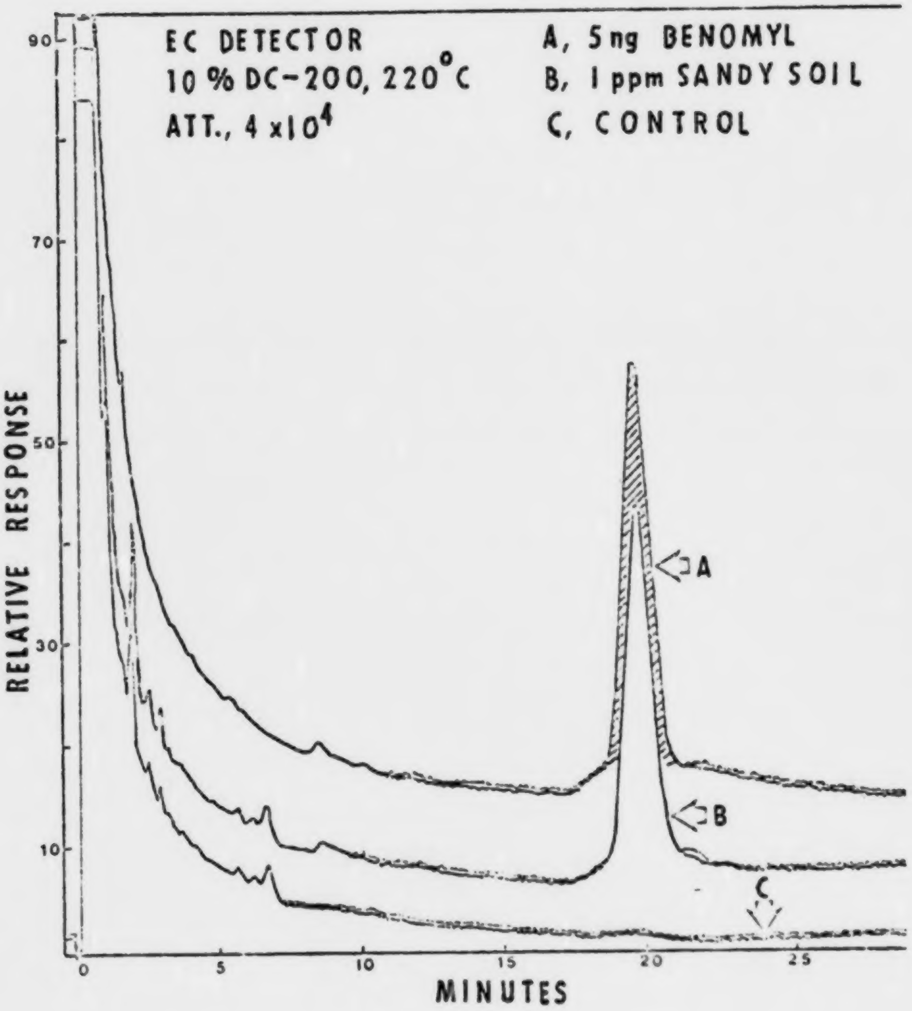
Figure 11


.0003% PYRIDINE
IN
BENZENE
Δ 95°C, 30 MIN.

2–AB

In Figure 12 we have a sandy soil spiked with benomyl at the one ppm level, versus a five nanogram standard, which would be equivalent to one ppm. You can see that after having gone through the silica gel column there is a very good recovery. We didn't calculate that out, but it gives a nice, strong peak on a DC-200 column at about 20 minutes. We plan to work more with this reagent this coming year.

Figure 12



Rather than force you to undergo the reading of the data and all of these extraction efficiencies, I thought I would just summarize briefly. Using methanol benzene as extraction solvent in a Soxhlet extractor, we had poor recoveries for these materials: monuron, Furadan, Bux, Zectran, IPC, Temik or aldicarb, captan, folpet, Difolatan, and somewhat a low recovery -- it's 50 to 71 percent -- of trifluralin.

Obviously, the methanol in the methanol-benzene extraction solvent is reacting with the carbamates to give an transesterification product. If you redo the extractions in acetone, however, you're not much better off. With monuron you get 11 to 30 percent; Furadan, 50 to 60 percent; Bux, 40 to 64 percent; Zectran, still not detectable. If you use benzene in a tumbling fashion, you can obtain captan, folpet, and Difolatan in 75-100, 85-100 and 80-82 percent recoveries throughout the 0.01 to 10 ppm range.

In summary, the materials that we could not derivatize are consequently gas chromatographed. Zectran, Temik, amitrole, Avadex, Azodrin, benomyl -- all those are questionable -- diquat and ferbam, the latter two we had really no hope for in the beginning. We have extraction problems with monuron, Furadan, Zectran, IPC, and Temik or aldicarb.

I would like now to jump right ahead and, if there are no questions on these extraction studies, talk about something I think was a little bit more interesting, at least from our standpoint. We wanted to see if we could not flesh out the methodology available for carbamates since, as you know, they do decompose quite quickly under gas chromatographic conditions if they are not derivatized. There have been reported some techniques whereby large amounts of injected sample have been satisfactory; however, there is a problem in doing this with detectors, such as the microelectrolytic conductivity detector, of contamination of columns and furnaces and things of that nature.

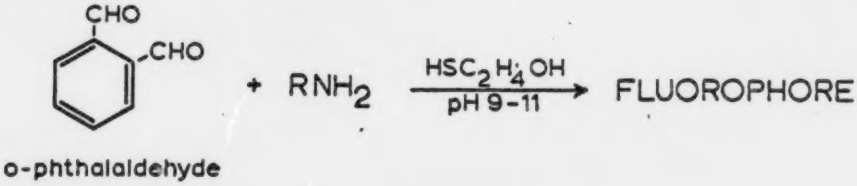
We have been working with what we call a dynamic fluorogenic labeling detector. This is a device that is based around the idea that a high-speed liquid chromatographic separation can be achieved, and subsequent to the column a reaction can be made to occur that will convert a nonfluorescent material to a highly fluorescent material. The basis of this revolves around two fundamental points. One is that you can find a liquid phase in a column that will be compatible with a reaction that will take place to give a fluorometric response. The other is that you can find the reagent that will give a fluorometric response that has an almost instantaneous reaction and also has by-products and beginning products which do not themselves fluoresce and therefore interfere with the materials under investigation.

We looked at a number of reagents, some on paper, two in the laboratory. We spent almost all the contract year on one and essentially ran into a dead end. It is a reagent called fluoram or fluorescamine, which was developed at Hoffman-LaRoche. It's a reagent that is specific for primary amines. This materials reacts in milliseconds under pH 9 aqueous conditions at room temperature with primary amines to give a very strong fluorophore. Fluoram itself does not fluoresce, nor do the hydrolytic products which occur upon the exposure to pH 9 conditions. The reaction product is the only fluorescent material.

This reagent had been picked up by American Instrument Company and made as the heart of their amino acid analyzer. They had been working with it for about a year, and during the course of our studies we consulted them and discovered that they had arrived at the same conclusions we had, that is, that the requirement that fluoram be dissolved in an aprotic organic solvent was such that when it was mixed with the pH 9 aqueous buffer which had to be added later (that is, you could not mix or you could not add the fluoram to the buffer and have that react with your eluting compound) it caused extreme noise in the flow through cell and fluorometer due to inhomogeneous mixing. We went through a whole series of mixing devices, heating devices, tumbling devices, and found out that even after pumping in acetone into a pH 9 buffer and giving as much as two minutes for mixing through these various devices, we could not get rid of the noise. American Instrument Company discovered this also, and they have now issued a disclaimer to this reagent as a derivatization reagent for amino acids.

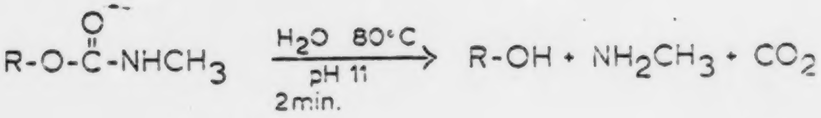
One reagent with which we did work which was surprisingly productive was orthophthalaldehyde. This is a reagent which has been around a while. There was a publication in 1971 by Mark Roth from a hospital in Geneva, Switzerland, where he reported that if you mix this reagent in the presence of mercaptoethanol under conditions about pH 9 to 11, you get an extremely strong fluorophore. This again is a millisecond, if not a second or so reaction (Figure 13).

Figure 13



After looking at this reaction in a static system, we went to a dynamic flow scheme whereby we based our whole arrangement on the capability of methyl carbamates to be hydrolyzed under very mild conditions quite rapidly (Figure 14). This was quite surprising. We knew that they could be hydrolyzed to give a phenolic or hydroxyl product plus methylamine plus CO_2, but we were surprised at how readily this could be made to occur; pH 11 is a somewhat mild hydrolytic condition.

Figure 14



$$R\text{-}O\text{-}\overset{O}{\overset{\|}{C}}\text{-}NHCH_3 \xrightarrow[\substack{\text{pH 11} \\ \text{2 min.}}]{H_2O\ \ 80°C} R\text{-}OH + NH_2CH_3 + CO_2$$

Using this information, we set up the arrangement illustrated in Figure 15. The heart of the dynamic fluorogenic labeling detector instrument was, of course, the

Waters 6000 pump, which allowed separations to be obtained on a micro C18 reverse phase column, also obtained from Waters. I might say that many long hours went into selecting the right column for these carbamates. We examined essentially every adsorption, reverse phase and normal phase column, and even took a few pot shots at ion exchange columns, thinking that they might have some adsorptive properties which were somewhat rare, and arrived at this micro C18 column, which allowed us to perform a quite good separation for a number of N-methyl carbamates, using 40 percent acetonitrile in water.

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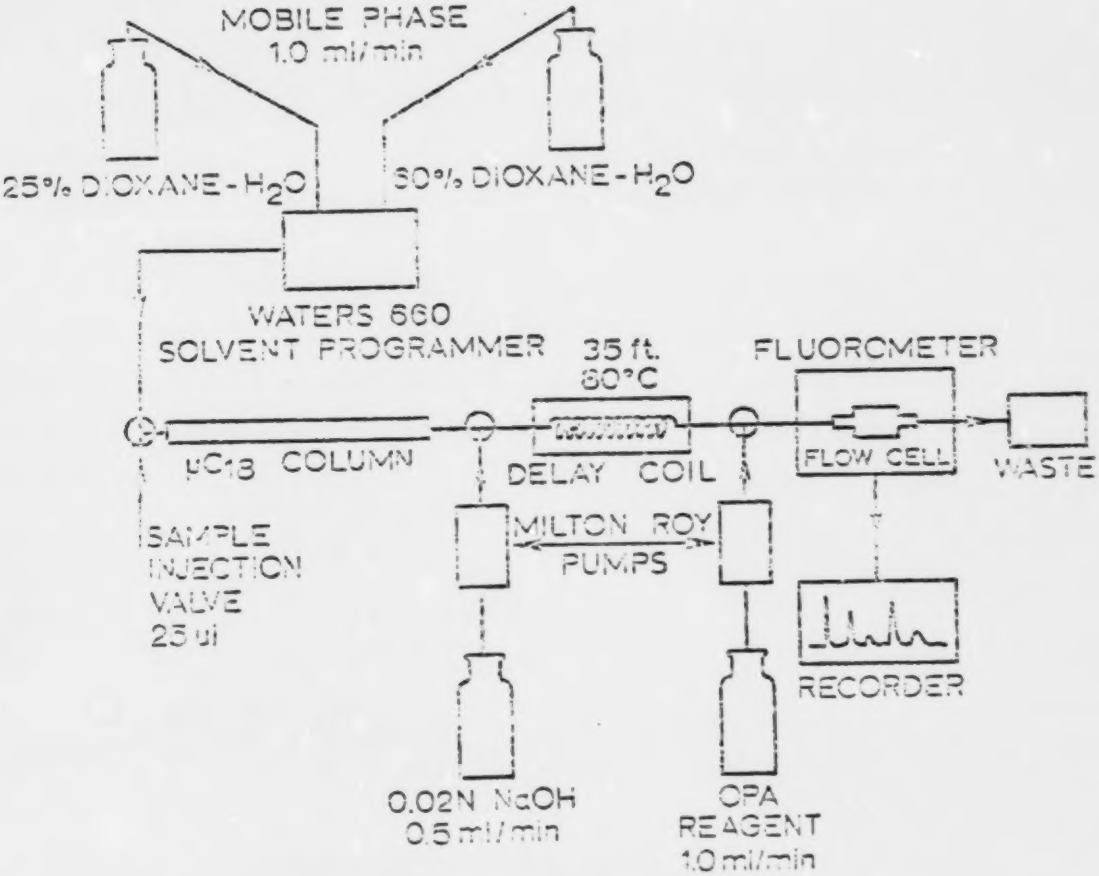
Figure 15


MOBILE PHASE
1.0 ml/min
25% DIOXANE - H_2O
80% DIOXANE - H_2O
WATERS 660
SOLVENT PROGRAMMER
35 ft.
80°C
FLUOROMETER
μC18 COLUMN
DELAY COIL
FLOW CELL
WASTE
SAMPLE
INJECTION
VALVE
25 ul
MILTON ROY
PUMPS
RECORDER
0.02N NaOH
0.5 ml/min
OPA
REAGENT
1.0 ml/min

I show dioxane there because we found that when we got the system going the dioxane was incompatible with the orthophthalaldehyde. Acetonitrile did give the best separations; however, it has a tendency to decompose and give ammonia, which reacts instantly with the orthophthalaldehyde and gives a high blank.

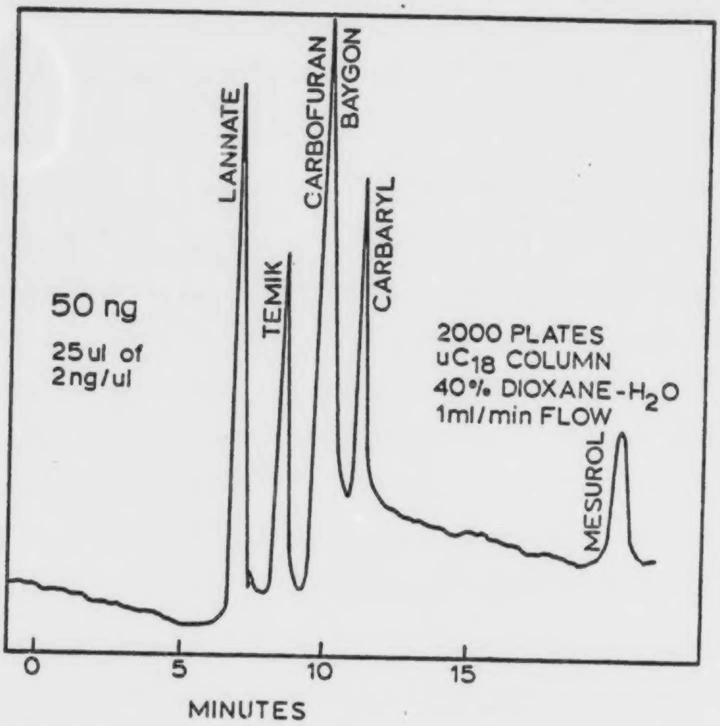
Going over to the dioxane, we lost a little bit of efficiency in the column, but it was exceptionally satisfactory in terms of signal to noise and response. After going through the column, there is a mixing whereby pH 11 borate buffer is metered in with the Milton Roy single piston displacement pump at a 0.5 ml per minute. It goes through a 35-foot mixing coil or reaction coil. This sounds almost impossible, but this was made 0.01 mm I.D. diameter. No bubbles were involved, as is the custom with Technicon; this was held to 80° C in a water bath, went through another mixing tee where orthophthalaldehyde reagent, along with the mercaptoethanol, was metered in at a milliliter per minute. The whole system is of Teflon; all of these tees and the tubing are of Teflon.

It goes directly, with no delay, through a flow-through fluorometric cell that was 5 mm^2 on a side. We have a photometer and a readout on the recorder and then to waste.

Figure 16 is a chromatogram of a mixture of five carbamates. Although Lannate and Temik are really not carbamates, we always throw them in. They do give methylamine upon the hydrolysis conditions that we have set down. Observe the relatively sharp peak shape. We're talking about 50 nanograms per compound. There is a 25 microliter sample injection. This is in water with a small amount of dioxane, a few percent dioxane for solubilization. Our experiments have shown that this can be boosted to as much as 250 microliters -- that's a quarter of a millimeter -- on a one-foot analytical column without any degradation in efficiency.

This means that you can use very dilute samples and, therefore, theoretically approach very low limits of detection. This arrangement -- notice Lannate comes

Figure 16

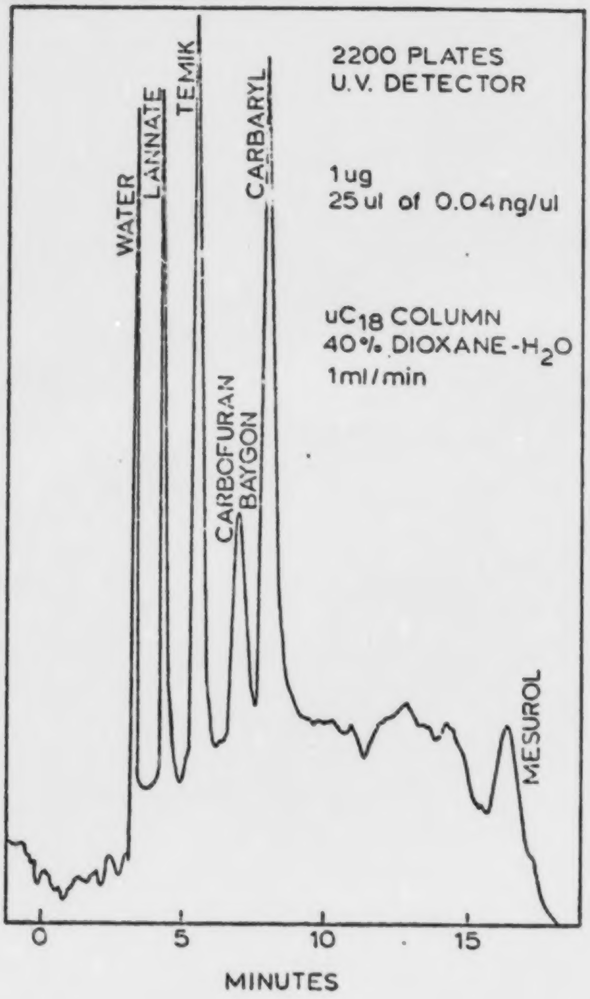


out in about 7 minutes -- gives 2,000 theoretical plates, calculated on Lannate. The same solution injected upon the same column but with the Chromatronics 254 nanometer mode UV adsorption detector, at 0.02 adsorbence units, gives 2,200 plates (Figure 17). So we're talking about just a 10 percent increase in separatory power.

Notice the additional noise at one microgram for each sample with the 25-microliter injection. We were able to run just a few analytical curves (Figure 18). There is an almost linear response for Lannate from one nanogram up to 150 nanograms. Baygon and Temik also give somewhat linear analytical curves.

The limit of detection is approached at around one nanogram. Figure 19 shows Lannate in an analytical standard. It's five or six times the noise. The proof of

Figure 17



the pudding is in the selectivity. We spiked sandy soil, the soil you saw before, with crude extracts, getting the large interfence peaks by DC-200 (Figure 20). There's nothing there. You get no solvent response. This bump is kind of an artifact in a detector; 0.05 ppm corresponds to 125 nanograms. There are really about three peaks, and these peaks seem to come and go in spiked soil samples, which make me think that there is some sort of decomposition going on. You can see the 0.5 ppm is way off scale.

Figure 18

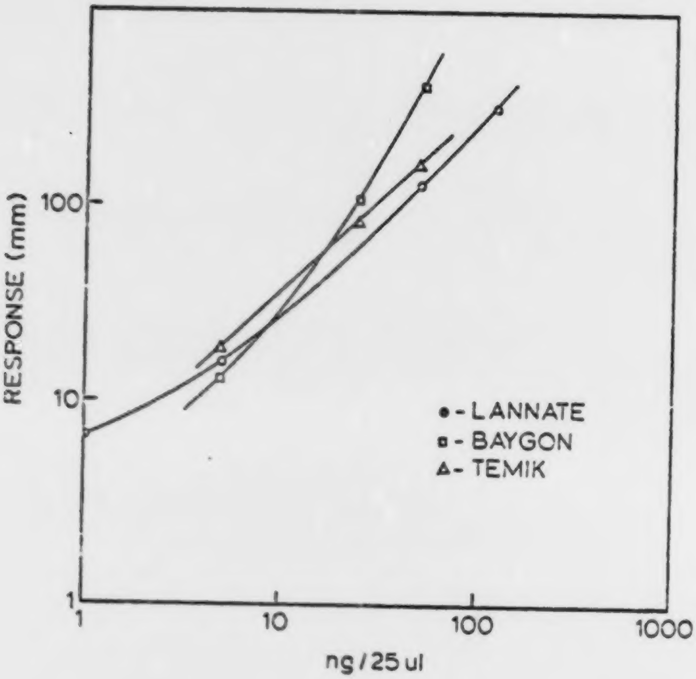


Figure 19

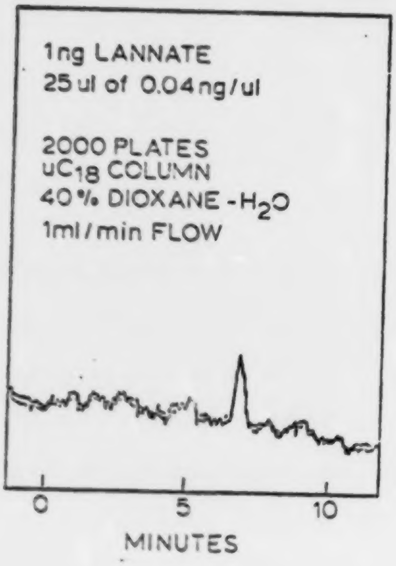
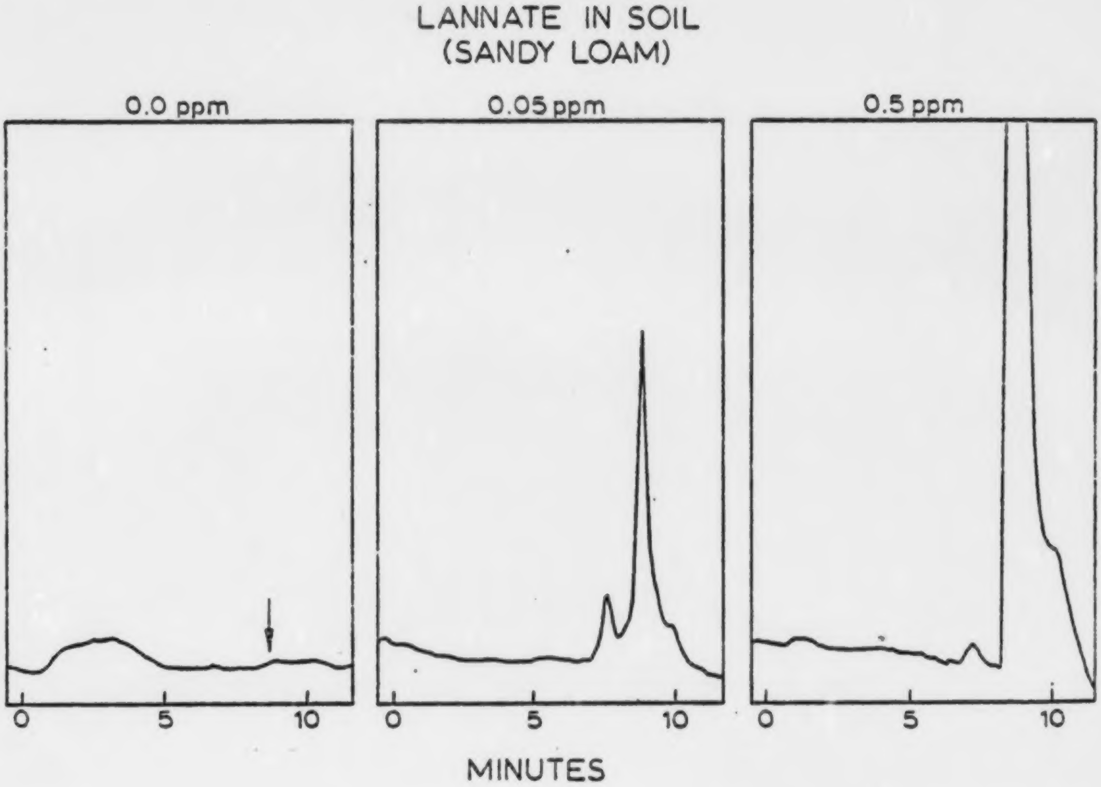


Figure 20

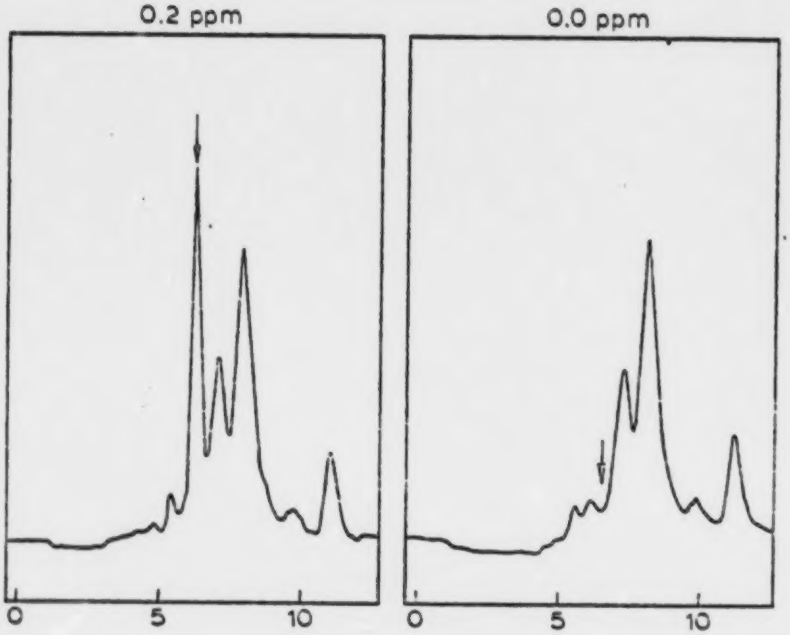


This is without any cleanup at all. Acetone was used to extract the soil. It was evaporated off and then the residue picked up in water with a small percentage of dioxane for solubilization. We spiked lettuce with Lannate, refluxed it with 0.25 normal sulfuric acid for a half an hour, partitioned that into methylene chloride, concentrated it down, and shot the raw extract; it was green (Figure 21). You can see the control. There is a kind of a hole there; 0.2 ppm shows up quite nicely.

I might add that you just don't go injecting these raw extracts on an analytical column without some sort of treatment. They were put through millipore filters to rid them of suspensions of all particulate matter.

Figure 21

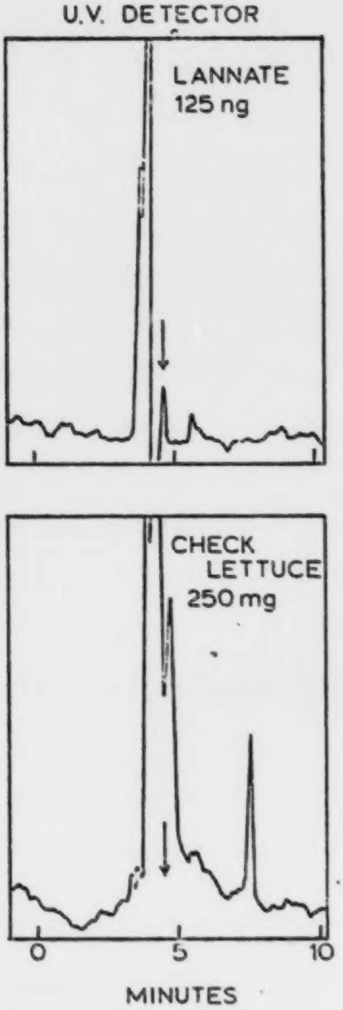
LANNATE IN LETTUCE



MINUTES

Figure 22 shows the same sample injected on a UV detector. Notice the 125-nanogram standard which is equivalent to 0.05 ppm falls here in about 5 minutes, which is completely obscured by interferences on 0.02 adsorbence unit scale of the UV detector. I think we have the basis with this device for doing some very good separations and some high-sensitivity detections of these carbamates. I might add that we have not done extensive interference studies. We do know that N-phenyl carbamates do not either hydrolyze or give the response, so we've run things like IPC and CIPC and obtained nothing on this detector.

Figure 22



We also know that substituted urea herbicides under these conditions give no response, so it is fairly specific. That, in a nutshell, has been our effort for this past year on the contract extension of multiresidue methodology and, I suppose, tomorrow you will hear some plans for the coming year on this contract.

DR. ZWEIG: Do you believe that most of the N-methyl carbamates that you've tested will undergo this hydrolysis reaction, if you can call it such, under these conditions, pH 11, and the second point is what do you think is the by-product that you seem to find in the small peak that you get there?

DR. MOYE: All of the N-methyl carbamates, and I say N-methyl carbamates, will hydrolyze under these conditions almost 100 percent. We're talking about 80 to 100 percent. The by-products, I don't know. There's something in the soil (we were spiking the soil and immediately extracting it in just a few minutes). It was dirty soil, if you will; it was not sterilized. It had a high microbial population, a high organic content. So there was something going on apparently between the soil and, in this case, Lannate to give some peaks that were not there in either the check soil or the Lannate standards, and I cannot even guess what those products might be.

DR. ZWEIG: What is your retention in the detector and the flow rate of solvent?

DR. MOYE: The delay in the 35-foot length of Teflon tubing is two minutes and there is essentially no bandspring, very, very little.

DR. HILL: I know you'd rather talk about the fluorogenic labeling, but I'd like to go back to an earlier part of your presentation. On the slide you showed on the various fractions of the silica gel cleanup, what were the volumes of eluant you were getting for each fraction? I didn't see that on the slide, or maybe I missed it.

DR. MOYE: That's a good question. We eluted each fraction with 50 milliliters.

DR. HILL: These were essentially uniform or equal fractional volumes for --

DR. MOYE: They were equal.

DR. HILL: You were changing the polarity with the mixtures.

DR. MOYE: Right. They were equal volumes, and they were concentrated down to a volume of one milliliter, so that 0.01 ppm of the pesticides that were noted on the chromatograms would give a measurable response. In other words, under the conditions of that separation in the 40 ml elution volume 0.01 ppm was detectable. That's all I can say.

DR. HILL: And then on the next set of slides where you showed the chromatograms of the various soil extract fractions, was that the same detector used for each of those or --

DR. MOYE: It was electron capture.

DR. HILL: It was EC for all of those?

DR. MOYE: Yes.

DR. ROSS: You mentioned silica gel. Did you look at any other column types? For example, your Fisher aluminum, Woelm, and so forth?

DR. MOYE: No, we didn't.

DR. ROSS: And the consistency of separation per class of compounds for these?

DR. MOYE: No, we did not.

DR. ROSS: In other words, this was the only procedure you chose. You just took the silica gel procedure as published by Shafik and Sherma and tried that!

DR. MOYE: Yes. We scaled it up somewhat. They were using it to separate materials in air, trapped air samples, and they had a microcolumn arrangement where they used, if I recall, about one gram in a five millimeter diameter chromatographic column. We used the two and a half centimeter, which required scaling up to 16 grams of silica gel and a subsequent increase in the volume of solvent.

DR. ROSS: What do you predict as far as enhancing sensitivity in the interfacing of the LC with the fluorometer system?

DR. MOYE: Maybe I didn't understand that. Would you repeat that?

DR. ROSS: Do you predict anything in the future about the enhancing sensitivity for this particular type of detector system?

DR. MOYE: For what particular compounds or any of them?

DR. ROSS: Any of them in the group or a particular class, particularly with the heat labile compounds.

DR. MOYE: We are able to see one nanogram of N-methyl carbamates. The people at American Instrument Company are seeing about 0.01 nanogram of amino acid, using this orthophthalaldehyde. So we're at about a hundred-fold difference somewhere along the line, and I don't think we're going to pick up that additional sensitivity by fine tuning the reagents and the conditions of the reaction. I don't know what the difference in sensitivity is, whether it's due to the inherent response of amino acids versus methyl-amine or not, but the point is that with this limit of sensitivity for the N-methyl carbamates and the large injection volumes that can be handled under these reverse phase conditions on the analytical column, there is no bottom to the limit of detection, I think, in terms of parts per million from samples like soil in particular and water in particular.

IDENTIFICATION OF IMPURITIES IN TECHNICAL-GRADE PESTICIDES

Dr. J. S. Warner*

During the past year, Battelle's Columbus Laboratories have been involved in a program aimed at identifying impurities in some of the candidate substitute pesticides. The objective is to acquire enough information about what's present to avoid the possibility of selecting a substitute pesticide which has some toxic impurity that makes the substitute material as hazardous or more hazardous than the material it is intended to replace. Perhaps the most dramatic example of a toxic impurity is the tetrachlorodibenzodioxin found in 2,4,5-T. This impurity, which went undetected for many years, is so extremely toxic, teratogenic, and mutagenic that its presence in the technical-grade pesticide must be kept below one ppm. Naturally, we would like to know whether any candidate substitute pesticide contains any unknown contaminant having a toxicity similar to that of tetrachlorodibenzodioxin.

The task of identifying every impurity present in each technical-grade pesticide and determining the biological effects of those impurities is a monumental task indeed. From a practical standpoint, it may well be an impossible task. Nevertheless, there is merit in studying candidate substitute pesticides to obtain as much information as reasonably possible about impurities present and their potential hazards.

There are at least three possible approaches to studying the impurities present in technical-grade pesticides. One approach involves studying the manufacturing process, identifying the main reactions and the numerous possible side reactions that may occur, listing the potential impurities that may result, and estimating the the likelihood of their actually being present at ppm or even subppm levels in the final product. A second approach involves taking an actual sample of the technical-grade pesticide and subjecting it to the many separation and analysis schemes available

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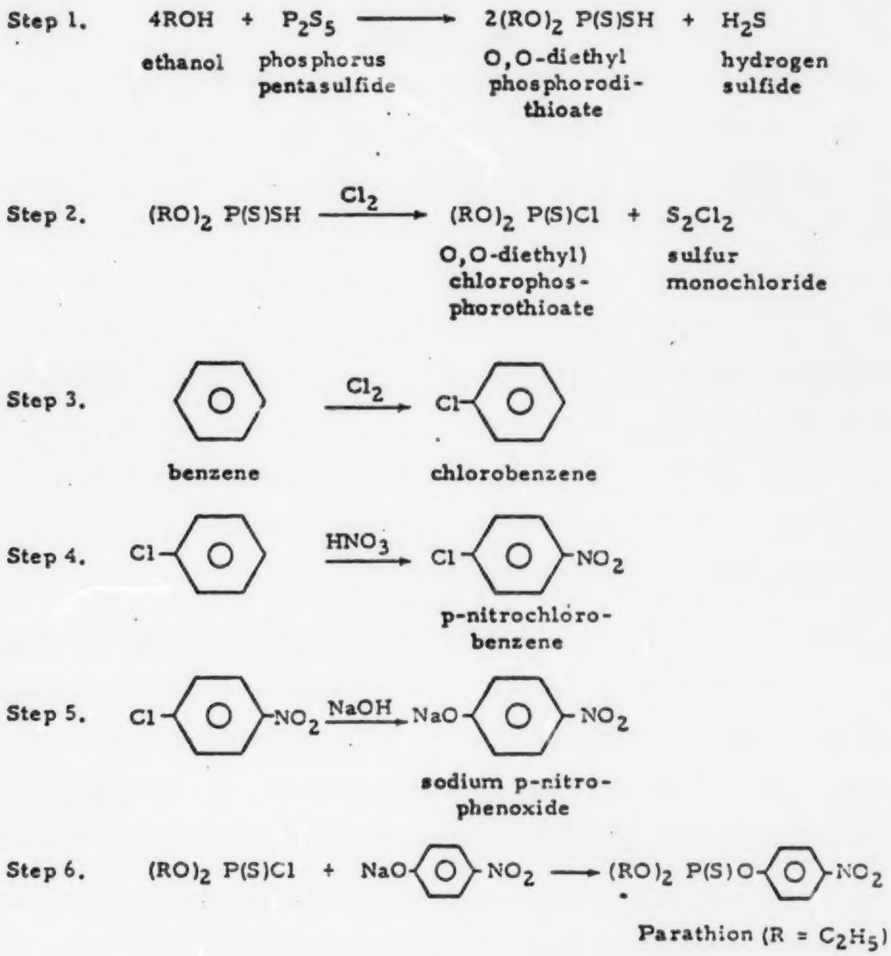
*Battelle's Columbus Laboratories, Columbus, Ohio

Figure 1. Main Reactions in the Preparation of Parathion


Step 1.
4ROH + P2S5 → 2(RO)2 P(S)SH + H2S
ethanol
phosphorus pentasulfide
O,O-diethyl phosphorodithioate
hydrogen sulfide
Step 2.
(RO)2 P(S)SH →Cl2 (RO)2 P(S)Cl + S2Cl2
O,O-diethyl) chlorophosphorothioate
sulfur monochloride
Step 3.
Cl2
Cl
benzene
chlorobenzene
Step 4.
Cl
HNO3
Cl
NO2
p-nitrochlorobenzene
Step 5.
Cl
NO2
NaOH
NaO
NO2
sodium p-nitrophenoxide
Step 6.
(RO)2 P(S)Cl + NaO
NO2
(RO)2 P(S)O
NO2
Parathion (R = C2H5)

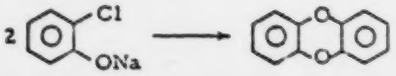
to researchers today. A third approach, and certainly a potentially far more cost effective approach, involves acquiring the existing information on pesticide impurities that has accumulated over the years by the various pesticide manufacturers.

The program at Battelle has involved a combination of the first two approaches. The potential contaminants suggested by possible side reactions occurring during production and storage were used as a guide in identifying impurities detected by a rather cursory gas chromatographic-mass spectrometric (GC-MS) analysis.

Table 1: Reactions Occurring During Production of Parathion

Reaction Number	Generalized Reaction(a)
1.	$4ROH + P_2S_5 \rightarrow 2(RO)_2P(S)SH + H_2S$
2.	$(RY)_2P(Y)SH + R'OH \rightarrow (RY)_2P(Y)SR' + H_2O$
3.	$(RY)_2P(Y)SH + R'OH \rightarrow (RY)_2P(Y)OR' + H_2S$
4.	$(RY)_2P(Y)SR + R'OH \rightleftharpoons (RY)_2P(Y)OR' + RSH$
5.	$(RY)_2P(Y)OR \xrightarrow{P_2S_5} (RY)_2P(Y)SR$
6.	$(RY)_2P(Y)SH + H_2O \rightarrow (RY)_2P(Y)OH + H_2S$
7.	$(RY)_2P(S)OH \rightleftharpoons (RY)_2P(O)SH$
8.	$(RY)_2P(Y)YH \rightarrow (RY)_2P(Y)YP(Y)(RY)_2 + H_2Y$
9.	$(RY)_2P(Y)SH \xrightarrow{[O]} (RY)_2P(Y)SSP(Y)(RY)_2$
10.	$(RY)_2P(S)OR' \rightleftharpoons (RY)_2P(O)SR'$
11.	$(RY)_2P(S)SH \rightarrow (RY)_2PSH + S$
12.	$(RY)_2PSH + R'OH \rightarrow (RY)_2POR' + H_2S$
13.	$(RY)_2PSH + R'OH \rightarrow (RY)_2PSR' + H_2O$
14.	$(RY)_2PSH \xrightarrow{[O]} (RY)_2P(S)SP(YR)_2$
15.	$(RY)_2PSH + Cl_2 \rightarrow (RY)_2P(S)SP(YR)_2 + 2HCl$
16.	$(RY)_2P(S)SH + Cl_2 \rightarrow (RY)_2P(S)SSP(S)(YR)_2 + 2HCl$
17.	$(RY)_2P(S)SSP(S)(YR)_2 + Cl_2 \rightarrow 2(RY)_2P(S)Cl + S_2Cl_2$
18.	$2S_2Cl_2 + 2H_2O \rightarrow SO_2 + 4HCl + S$
19.	$ArH \xrightarrow{Cl_2} ArCl$
20.	$ArH \xrightarrow{HNO_3} ArNO_2$
21.	$ArCl \xrightarrow{NaOH} ArONa \xrightarrow{H^+} ArOH$

Table 1. (Continued)

Reaction Number	Generalized Reaction[a]
22.	$ArCl + ROH \longrightarrow ArOR + HCl$
23.	$ArCl + ArONa \longrightarrow ArOAr + NaCl$
24.	 + 2NaCl
25.	$(RY)_2P(Y)Cl + ArONa \longrightarrow (RY)_2P(Y)OAr + NaCl$
26.	$(RY)_2P(Y)OAr + ArONa \longrightarrow (RY)(ArO)P(Y)OAr + RONa$
27.	$(RY)_3PY + ArONa \longrightarrow (RY)_2P(Y)YNa + ArOR$
28.	$(RY)_2P(Y)Cl + (RY)_2P(Y)YNa \longrightarrow (RY)_2P(Y)YP(Y)(YR)_2 + NaCl$
29.	$(RY)_2P(Y)Cl + ROH \longrightarrow (RY)_2(OR)PY + HCl$
30.	$(RY)_3PS \xrightarrow{[O]} (RY)_3PO$
31.	$(RY)_3PY + H_2O \longrightarrow (RY)_2P(Y)OH + RYH$
32.	$RSH \xrightarrow{[O]} RSSR$

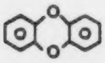
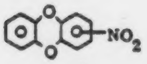
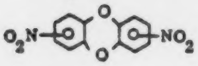
(a) R = alkyl or aryl group; Ar = aryl group; Y = oxygen or sulfur.

The identification of impurities present in technical-grade parathion will serve as an example. The manufacturing process is outlined in Figure 1. Steps 1 and 2 for the preparation of a chlorophosphorodithionate can potentially give rise to a myriad of exchange and disproportionation reactions that are typical of organophosphorus and sulfur chemistry. Steps 3 to 5 for the preparation of the p-nitrophenoxide are included because of the various chlorinated, nitrated, or phenolic compounds that may be formed by side reactions. A list of the various generalized reactions that may occur during production and storage of parathion are shown in Table 1. In addition to the main intermediates, the reactions can give rise to all types of phosphate esters, phosphites, pyrophosphates, ethers, and even dibenzodioxins. A list of many of the possible contaminants that could potentially be formed by these reactions is given in Table 2.

Table 2: Possible Contaminants in Parathion

No.	Contaminant[a]	Precursor[b]	Reaction Number[c]	Step[d]	Likelihood of Being Present in Final Product[e]
1	P_2S_5	SM	—	1	o
2	$(RO)_2P(S)SH$	1	1	1	o
3	$(RO)_2(RS)PS$	2 19	2 29	1 6	 ++
4	$(RO)_3PS$	2 3 18	3 4 29	1 1 6	++
5	$(RO)(RS)_2PS$	3	5	1	++
6	$(RO)(RS)P(S)SH$	2	5	1	o
7	$(RO)_2P(O)OH$	7	6	1	o
8	$(RO)_2P(O)SH$	2	6 + 7	1	o
9	$(RO)(RS)P(O)SH$	6	6 + 7	1	o
10	$(RO)_2P(S)SP(S)(OR)_2$	2	9	1	++
11	$(RO)_2P(S)SSP(S)(OR)_2$	2 2	10 16	1 2	++
12	$(RO)_2PSH$	2	11	1	o
13	$(RO)_3P$	12	12	1	+
14	$(RO)_2(RS)P$	12	13	1	+
15	$(RO)_2P(S)SP(OR)_2$	12 12	14 15	1 2	o
16	$(RO)_2(RS)PO$	4 9 21	10 3 29	1 1 6	 ++
17	$(RO)_3PO$	16 20	4 29	1 6	++
18	$(RO)_2P(S)Cl$	11	17	2	o
19	$(RO)(RS)P(S)Cl$	6	16 + 17	2	o
20	$(RO)_2P(O)Cl$	8	16 + 17	2	o
21	$(RO)(RS)P(O)Cl$	9	16 + 17	2	o
22	S_2Cl_2	11	17	2	o
23	S	22	18	2	++
24	C_6H_6	SM	—	3	o
25	C_6H_5Cl	24	19	3	o
26	$C_6H_4Cl_2$	25	19	3	o
27	$C_6H_4(NO_2)Cl$	24	20	4	+
28	$C_6H_3(NO_2)_2Cl$	27	20	4	o
29	$C_6H_3(NO_2)Cl_2$	26	20	4	o
30	$C_6H_2(NO_2)_2Cl_2$	29	20	4	o
31	$C_6H_4(OH)Cl$	26	21	5	o
32	$C_6H_4(NO_2)OH$	27 48	21 31	5 St	++
33	$C_6H_3(NO_2)_2OH$	28	21	5	+

Table 2: (Continued)

No.	Contaminant[(a)]	Precursor[(b)]	Reaction Number[(c)]	Step[(d)]	Likelihood of Being Present in Final Product[(e)]
34	$C_6H_3(NO_2)(OH)Cl$	29	21	5	o
35	$C_6H_2(NO_2)_2(OH)Cl$	30	21	5	o
36	$C_6H_4(NO_2)OC_6H_4NO_2$	27 + 32	23	5	+
37	$C_6H_3(NO_2)_2OC_6H_4NO_2$	28 + 32 27 + 33	23 23	5 5	o
38	$C_6H_3(NO_2)ClOC_6H_4NO_2$	29 + 32 27 + 34	23 23	5 5	o
39	$C_6H_4ClOC_6H_4NO_2$	26 + 32 27 + 31	23 23	5 5	o
40		31	24	5	o
41		31 + 34	24	5	o
42		34	24	5	o
43	$C_6H_4(OR)Cl$	26	22 or 27	6	o
44	$C_6H_4(NO_2)OR$	27	22 or 27	6	++
45	$C_6H_3(NO_2)_2OR$	28	22 or 27	6	+
46	$C_6H_3(NO_2)(OR)Cl$	29	22 or 27	6	o
47	$C_6H_2(NO_2)_2(OR)Cl$	30	22 or 27	6	o
48	$(RO)_2P(S)OC_6H_4NO_2$	18 + 32	25	6	Parathion
49	$(RO)_2P(S)OC_6H_3(NO_2)_2$	18 + 33	25	6	+
50	$(RO)_2P(S)OC_6H_3NO_2Cl$	18 + 34	25	6	o
51	$(RO)P(S)(OC_6H_4NO_2)_2$	48	26	6	+
52	$(RO)(RS)P(S)OC_6H_4NO_2$	19 + 32	25	6	++
53	$(RO)_2P(O)OC_6H_4NO_2$	20 + 32 48	25 30	6 St	++
54	$(RO)(RS)P(O)OC_6H_4NO_2$	21 + 32 48	25 10	6 6 or St	++
55	$(RO)_2P(O)SC_6H_4NO_2$	48	10	6 or St	+
56	$(RO)(NO_2C_6H_4O)P(S)OH$	48	31	St	o
57	$(RO)(NO_2C_6H_4O)P(O)OH$	56	7 + 6	St	+
58	$(RO)_2P(S)OH$	48	31	St	+
59	$(RO)_2P(O)OH$	58	7 + 6	St	++
60	RSH	3 or 52	31	St	+
61	RSSR	60	32	St	+

(a) $R = C_2H_5$

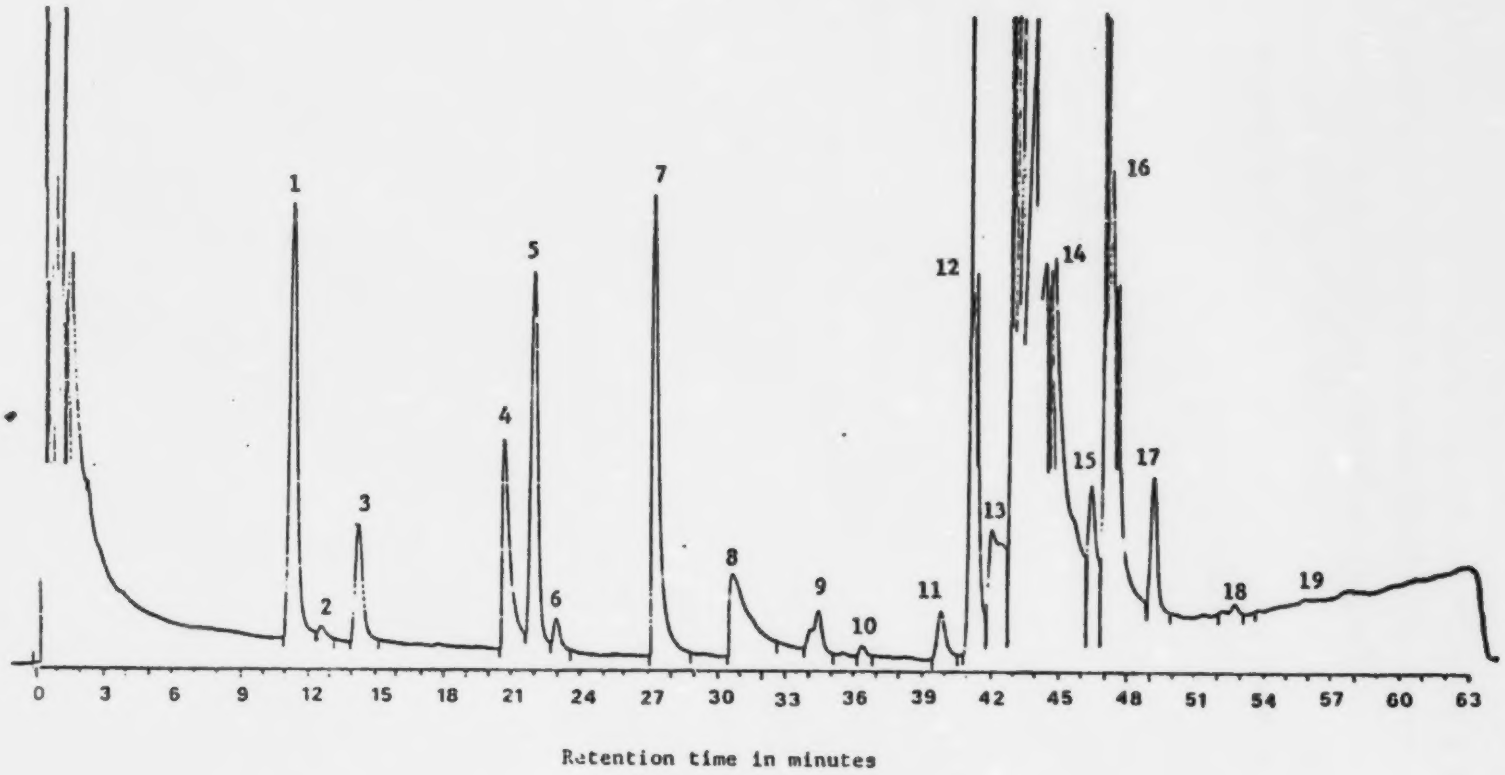
(b) Precursor refers to the contaminant numbered in this table. SM = starting material.

(c) See Table 1.

(d) The step (Figure 2) during which the contaminant might be formed. St indicates possible formation during storage.

(e) o = likely present only at levels below 1 ppm
+ = likely present at levels above 1 ppm but below 100 ppm
++ = likely present at levels above 100 ppm.

Figure 2. Gas Chromatogram of Technical-Grade Parathion



The next step was to obtain a gas chromatogram of an actual technical-grade sample. Since we were not interested in determining the amount of parathion present, but only interested in detecting as many impurities as possible, we used a very concentrated solution, 10 percent, and operated the gas chromatograph at as high a sensitivity as practical. The resulting gas chromatogram showing 19 components is given in Figure 2. A GC-MS run was then made in an effort to identify as many of these components as possible on a one-shot basis. The results are shown in Figure 3. We identified ten components, all of which were included in the list in Table 2. The phosphoryl chloride is an intermediate which we had expected would be more completely hydrolyzed. The three ethyl phosphates were expected as by-products from the reaction of P_2S_5 with ethanol. The p-nitrophenol intermediate was expected, of course, and the p-nitrophenetole was formed by reaction of the chloronitrobenzene with ethanol. One of the parathion isomers, probably the second one, is undoubtedly the thiolo compound. The first isomer may be an ortho-nitro compound. We did not have reference samples of the impurities or model compounds to permit us to determine which isomer was which. The dithio analog was also an expected impurity. The amounts of these impurities present range in general from a few percent to about

Figure 3. GC-MS of Technical-Grade Parathion

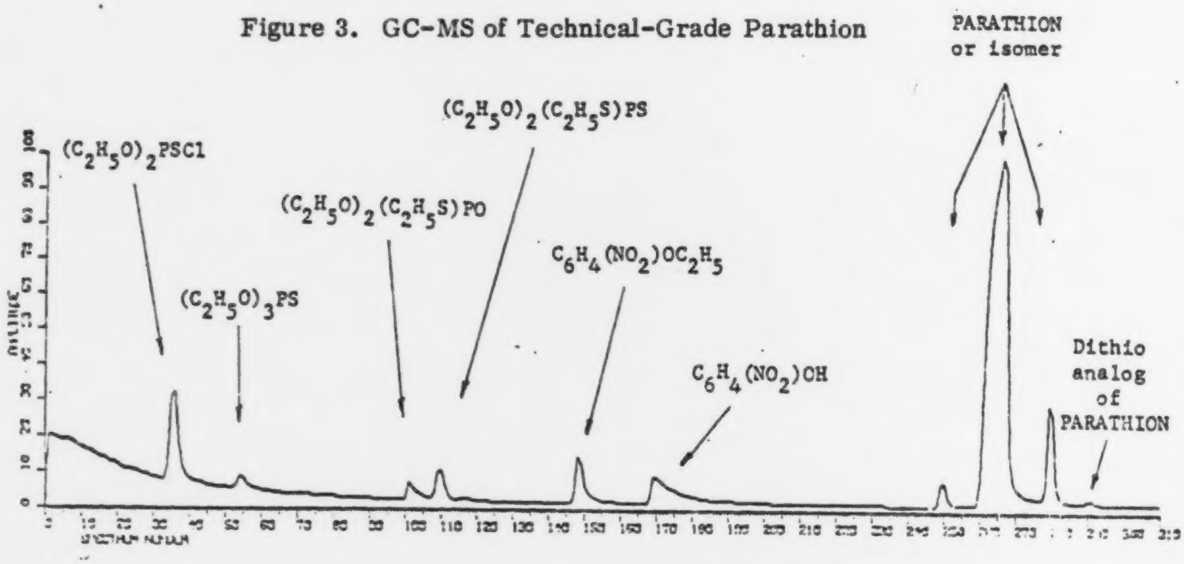
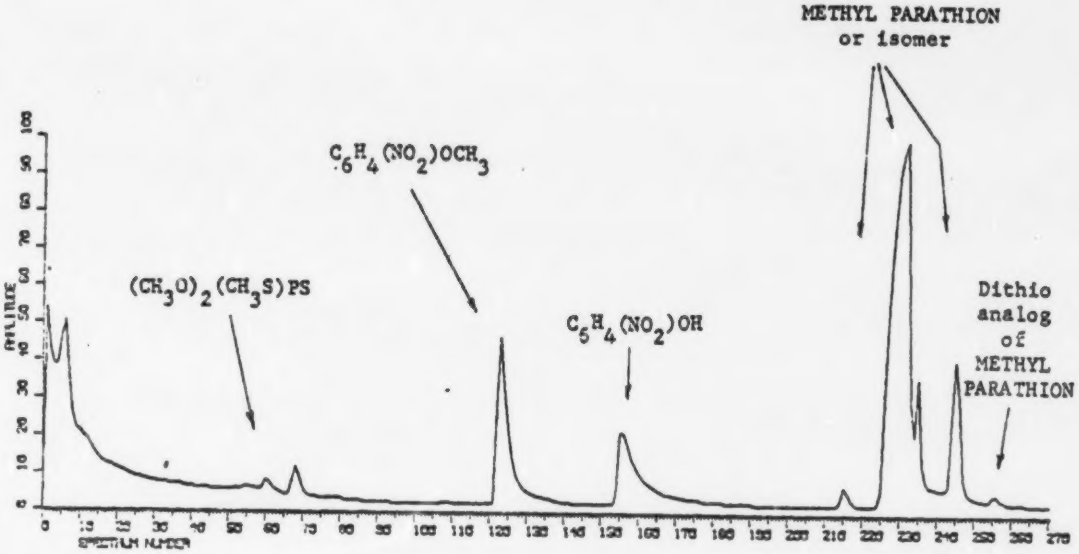


Figure 4. GC-MS of Technical-Grade Methyl Parathion

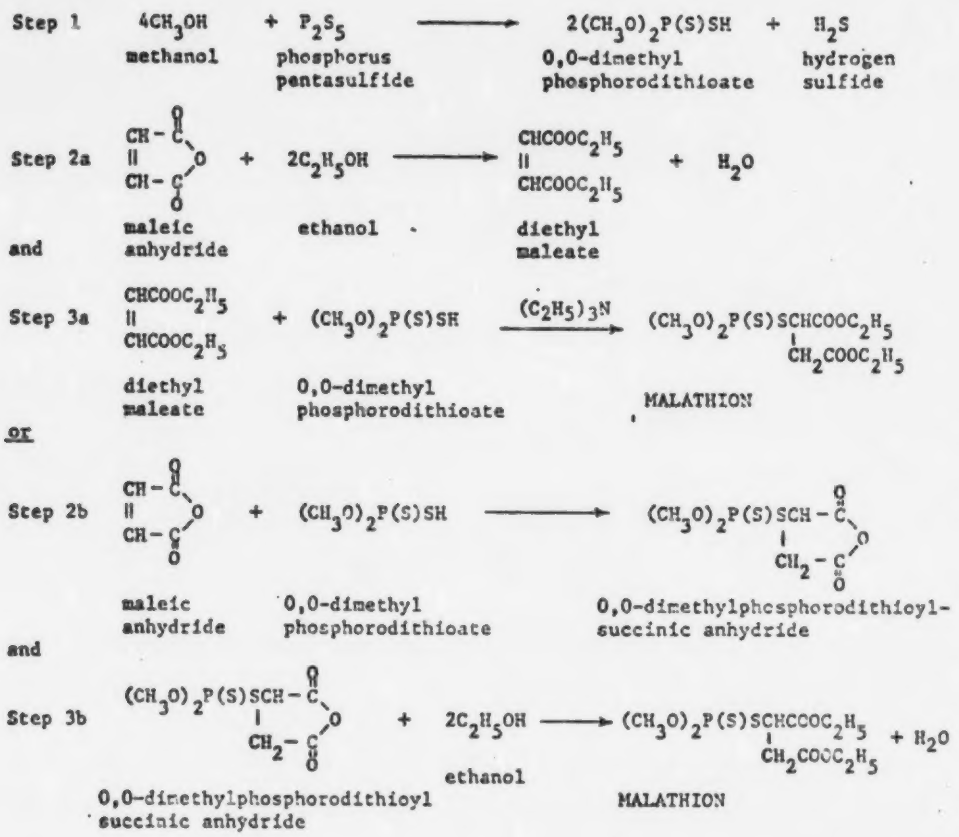


100 ppm. In this cursory study, no attempt was made to identify contaminants present at low ppm levels.

Similar results were obtained in the study of methyl parathion. The manufacturing process for methyl parathion, of course, is essentially the same as that for parathion except that methyl alcohol is used in place of ethyl alcohol. Seven of the impurities analogous to those found in parathion were identified. (See Figure 4.)

Another common organophosphate pesticide, malathion, is prepared by a somewhat different scheme as outlined in Figure 5. In this case, in addition to the various possible phosphorus-containing impurities, there is the very likely possibility of contamination by maleate intermediates and hydrogen sulfide or methyl mercaptan adducts. The GC-MS results are given in Figure 6. The eight components identified include two methyl phosphates, a pyrophosphate, diethyl maleate, and the hydrogen sulfide and methyl mercaptan adducts of diethyl maleate. The malathion isomer is again probably the thiolo isomer.

Figure 5. Synthesis of Malathion


Step 1
methanol
phosphorus pentasulfide
0,0-dimethyl phosphorodithioate
hydrogen sulfide
Step 2a
maleic anhydride
ethanol
diethyl maleate
and
Step 3a
diethyl maleate
0,0-dimethyl phosphorodithioate
MALATHION
or
Step 2b
maleic anhydride
0,0-dimethyl phosphorodithioate
0,0-dimethylphosphorodithioyl-succinic anhydride
and
Step 3b
0,0-dimethylphosphorodithioyl succinic anhydride
ethanol
MALATHION

The synthesis scheme for the organophosphate, phorate, also called Thimet, is given in Figure 7. In addition to the formation of the usual organophosphate by-products, it is quite likely that the hydroxymethyl intermediate will react with a second molecule of the dithioate to give a bis phosphoryl compound or react with the ethyl mercaptan in Step 3 with the loss of water instead of hydrogen sulfide and form the ethoxy analog. The GC-MS results are shown in Figure 8. Four ethyl phosphates were detected, a pyrophosphate, the ethoxy analog, and the bis phosphoryl compound.

Figure 6. GC-MS of Technical-Grade Malathion

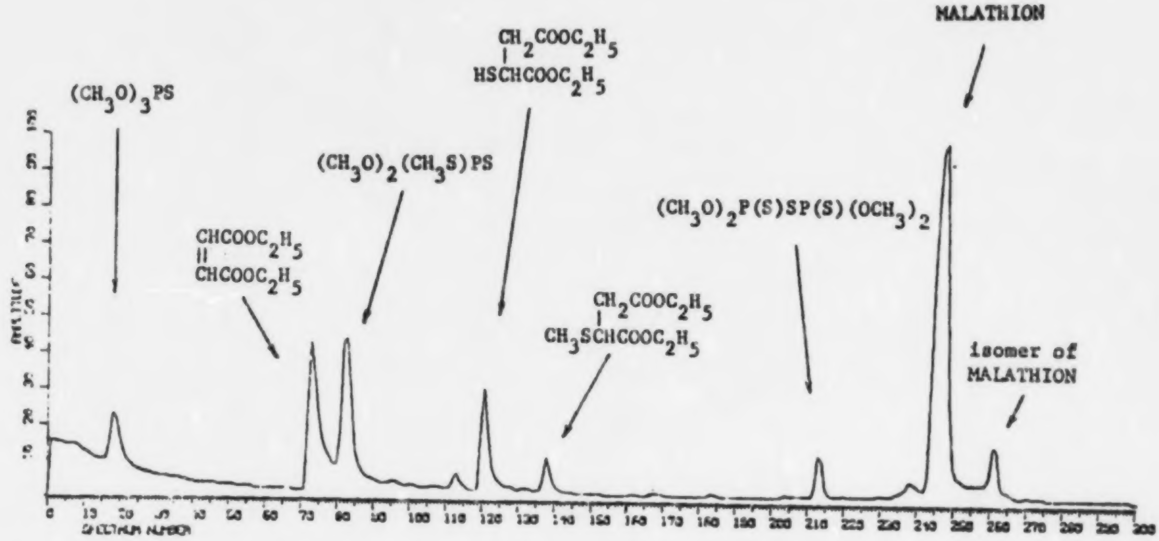


Figure 7. Synthesis of Phorate (Thimet)

Step 1 $4C_2H_5OH + P_2S_5 \longrightarrow 2(C_2H_5O_2)P(S)SH + H_2S$

ethanol; phosphorus pentasulfide; O, O-diethyl phosphorodithioate; hydrogen sulfide

Step 2 $(C_2H_5O)_2P(S)SH + HCHO \longrightarrow (C_2H_5O)_2P(S)SCH_2OH$

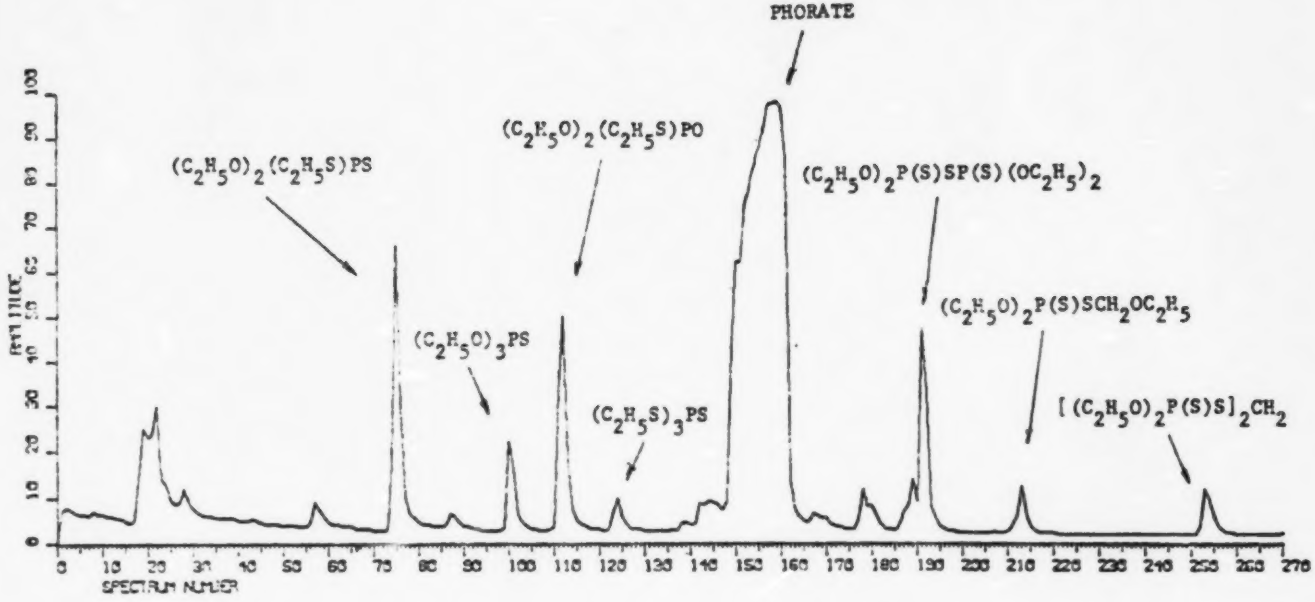
O, O-diethyl phosphorodithioate; formaldehyde; O, O-diethyl-S-(hydroxymethyl) phosphorodithioate (not isolated)

Step 3 $(C_2H_5O)_2P(S)SCH_2OH + C_2H_5SH \xrightarrow{50\ C} (C_2H_5O)_2P(S)SCH_2SC_2H_5 + H_2O$

O, O-diethyl-S-(hyroxymethyl) phosphorodiethioate; ethyl mercaptan; O, O-diethyl-S-(ethylthiomethyl) phosphorodithioate; water

PHORATE

Figure 8. GC-MS of Technical-Grade Phorate



A fifth organophosphate studied was Dursban (chlorpyrifos). The synthesis scheme is shown in Figure 9. An alternate method of preparing the intermediate 3,5,6-trichloro-2-pyridinol is shown in Figure 10. The various possible side reactions are listed in Table 3. In addition to the usual side reactions, there is the possibility of getting various substituted pyridines and also dipyridodioxins. The impurities actually identified by GC-MS are given in Figure 11. These include two ethyl phosphates, a pyrophosphate, the intermediate trichloropyridinol, and the tetrachloro analog of Dursban.

In addition to the above five organophosphates, we also studied some other classes of pesticides. One of these, PCNB, is one of the most pure pesticides available. The synthesis scheme is shown in Figure 12. The primary by-products expected are the various chlorinated and/or nitrated benzenes. It is also possible to get trace amounts of sulfonic acids, biphenyls, biphenylenes, dibenzodioxins, dibenzofurans, and diphenyl ethers. None of these latter possible contaminants were found. As shown in Figure 13, four different chlorinated and/or nitrated benzenes were found.

Another aromatic nitro compound studied, trifluralin, is prepared according to the scheme in Figure 14. Here there are possibilities for all sorts of aromatic by-products having chloro, fluoro, nitro, methylol, carboxyl, aldehyde, hydroxyl, ether, amino, and/or substituted amino functionalities. In fact, we came up with a list of 146 possible contaminants without taking into account positional isomers. The gas chromatogram (Figure 15) showed 14 components. In the GC-MS analysis, as shown in Figure 16, only two chloro compounds, in addition to four trifluralin isomers, were found.

The final pesticide that I'll report on is folpet. This material is prepared from phthalimide and perchloromethyl mercaptan according to the scheme in Figure 17. The various reactions that might occur during the production or storage of folpet are listed in Table 4. In addition to derivatives of phthalic anhydride, derivatives of maleic anhydride, and oxalic acid formed during the initial oxidation are possible. Various sulfenyl,

Figure 9. Synthesis of Dursban

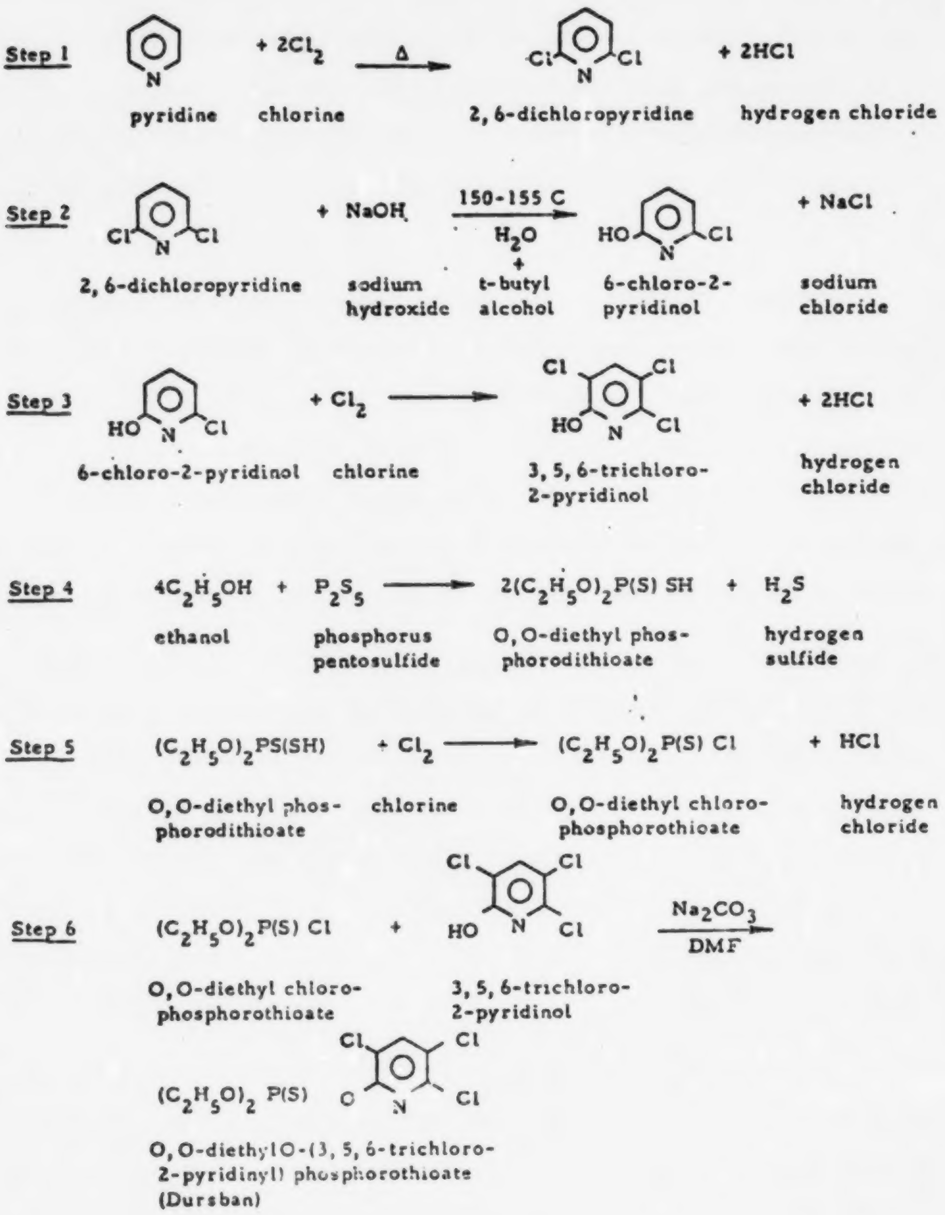
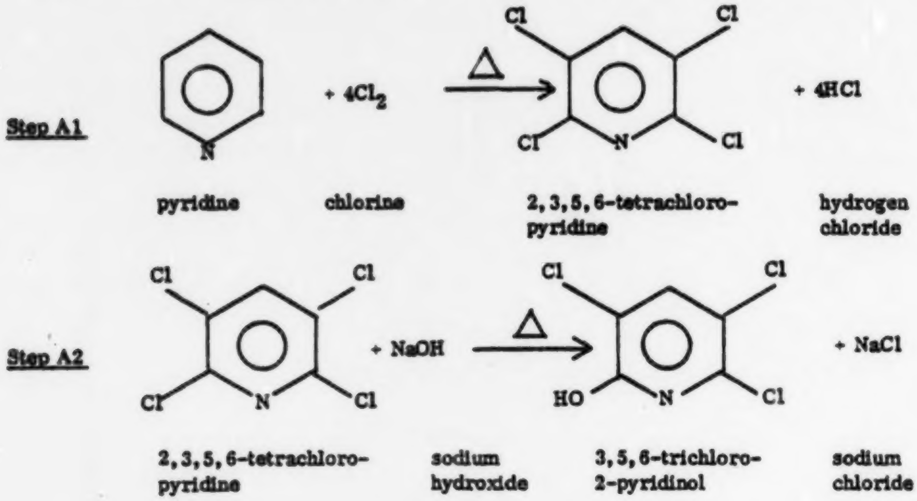

Step 1
N
+ 2Cl2
Δ
Cl N Cl
+ 2HCl
pyridine
chlorine
2, 6-dichloropyridine
hydrogen chloride
Step 2
Cl N Cl
+ NaOH
150-155 C
H2O
+
HO N Cl
+ NaCl
2, 6-dichloropyridine
sodium hydroxide
t-butyl alcohol
6-chloro-2-pyridinol
sodium chloride
Step 3
HO N Cl
+ Cl2
Cl Cl HO N Cl
+ 2HCl
6-chloro-2-pyridinol
chlorine
3, 5, 6-trichloro-2-pyridinol
hydrogen chloride
Step 4
4C2H5OH + P2S5
2(C2H5O)2P(S) SH + H2S
ethanol
phosphorus pentosulfide
O, O-diethyl phosphorodithioate
hydrogen sulfide
Step 5
(C2H5O)2PS(SH)
+ Cl2
(C2H5O)2P(S) Cl
+ HCl
O, O-diethyl phosphorodithioate
chlorine
O, O-diethyl chlorophosphorothioate
hydrogen chloride
Step 6
(C2H5O)2P(S) Cl
+
Cl Cl HO N Cl
Na2CO3
DMF
O, O-diethyl chlorophosphorothioate
3, 5, 6-trichloro-2-pyridinol
Cl Cl (C2H5O)2 P(S) O N Cl
O, O-diethyl O-(3, 5, 6-trichloro-2-pyridinyl) phosphorothioate
(Dursban)

Figure 10. Alternate Synthesis of 3,5,6-Trichloro-2-Pyridinol


Step A1
N
+ 4Cl2
Δ
Cl
Cl
Cl
N
Cl
+ 4HCl
pyridine
chlorine
2,3,5,6-tetrachloro-pyridine
hydrogen chloride
Step A2
Cl
Cl
Cl
N
Cl
+ NaOH
Δ
Cl
Cl
HO
N
Cl
+ NaCl
2,3,5,6-tetrachloro-pyridine
sodium hydroxide
3,5,6-trichloro-2-pyridinol
sodium chloride

sulfinyl, and sulfonyl impurities are possible, as well as acids, amides, imides, chloroamides, chloroinides, and maleic adducts. These are included in the list of possible contaminants shown in Table 5. The major contaminants found in the GC-MS study (Figure 18) were phthalic anhydride and phthalimide. The N-chloro-oxamic acid found was one of the least expected contaminants. We also found this compound in captan.

In conclusion, I want to emphasize again that so far we have only just skimmed the surface by identifying some of the more major impurities. Undoubtedly, most, if not all, of the impurities identified have been known to industry researchers for some time. There are undoubtedly hundreds of trace impurities which were not studied at all. The amount of work needed to identify all of the impurities that are present and may potentially be as hazardous as a tetrachlorodibenzodioxin is difficult to comprehend. A full man-year of effort on each pesticide could be very useful, but even then some of the lesser impurities would be missed.

Based on our experience so far in this program, several recommendations can be made. One recommendation, which I mentioned at the beginning, is to make as much use as possible of the information that has already been accumulated by the manufacturers and to build on that.

Figure 11. GC-MS of Technical-Grade Dursban

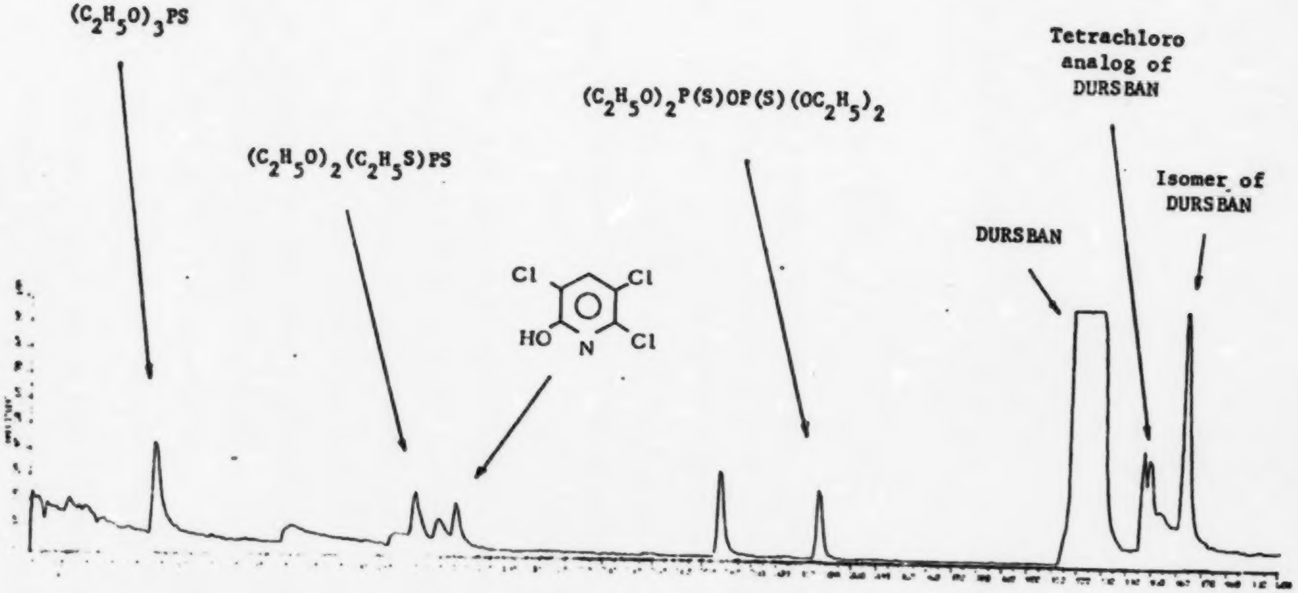
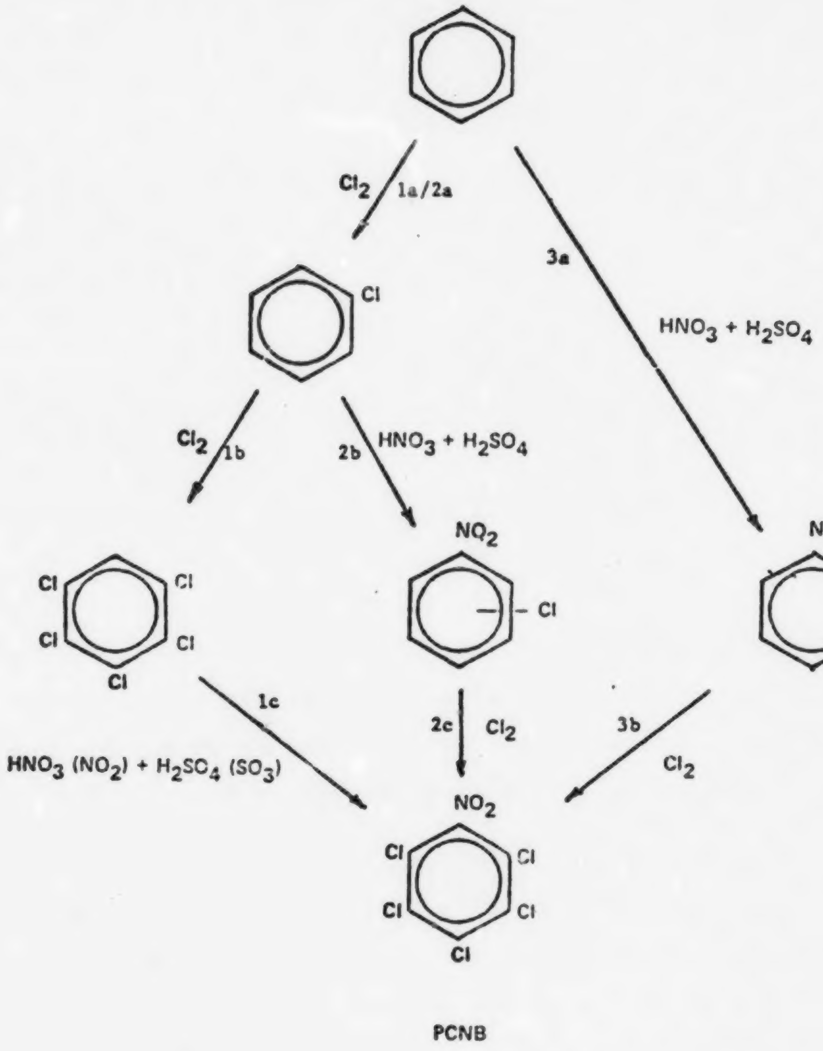
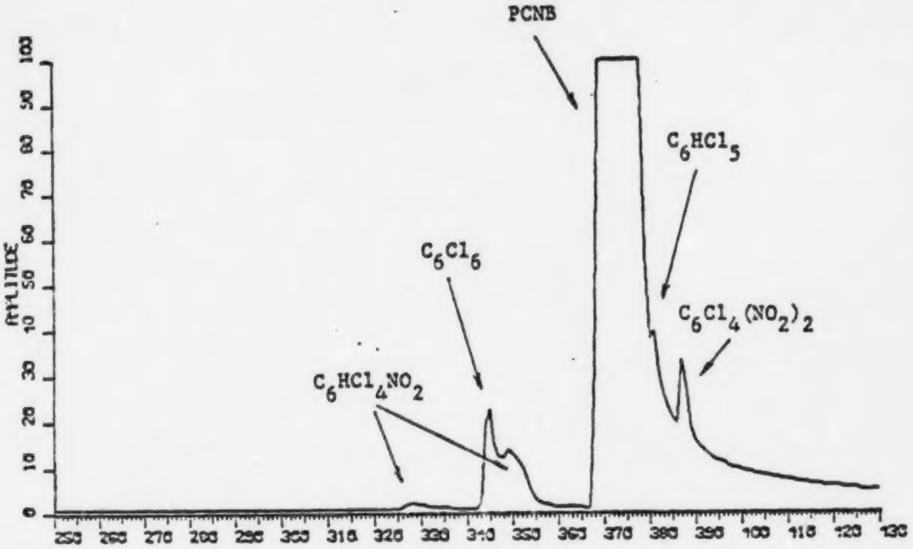


Figure 12. Three Methods for the Synthesis of PCNB



Cl_2
1a/2a
3a
$HNO_3 + H_2SO_4$
Cl
Cl_2
1b
2b
$HNO_3 + H_2SO_4$
NO_2
NO_2
Cl
Cl
Cl
Cl
Cl
Cl
1c
HNO_3 (NO_2) + H_2SO_4 (SO_3)
2c
Cl_2
3b
Cl_2
NO_2
Cl
Cl
Cl
Cl
Cl
PCNB

Figure 13. GC-MS of Technical-Grade PCNB



Another recommendation is that of making greater use of high-pressure liquid chromatography (HPLC) for the separation of impurities. By using HPLC, the less volatile and less thermally stable impurities, which may be overlooked in GC separation, can be readily isolated and studied by a variety of analytical methods.

A third recommendation is to use HPLC to obtain fractions for toxicological studies. In this way, a fraction containing a number of unknown components, each present at very low levels in the pesticide, might be shown to be harmless without requiring more extensive and costly analytical work.

One message that comes out of this work loud and clear is that a great deal more effort will be necessary before we can state with any degree of assuredness that substitute pesticide X contains no hazardous trace impurities.

Figure 14. Synthesis of Trifluralin

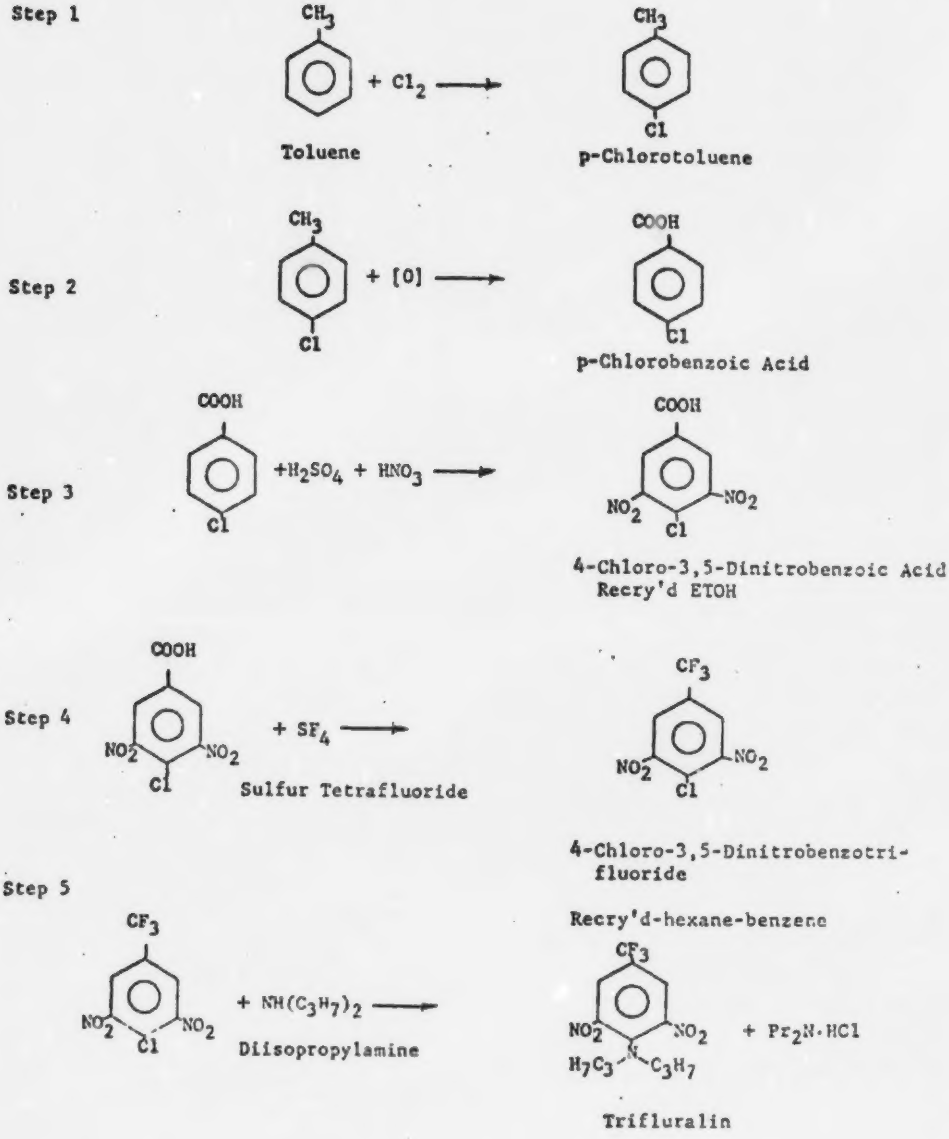

Step 1
CH_3
$+ Cl_2$
CH_3
Cl
Toluene
p-Chlorotoluene
Step 2
CH_3
+ [O]
COOH
Cl
Cl
p-Chlorobenzoic Acid
Step 3
COOH
$+H_2SO_4 + HNO_3$
COOH
Cl
NO_2
NO_2
Cl
4-Chloro-3,5-Dinitrobenzoic Acid
Recry'd ETOH
Step 4
COOH
NO_2
NO_2
Cl
$+ SF_4$
Sulfur Tetrafluoride
CF_3
NO_2
NO_2
Cl
4-Chloro-3,5-Dinitrobenzotrifluoride
Recry'd-hexane-benzene
Step 5
CF_3
NO_2
NO_2
Cl
$+ NH(C_3H_7)_2$
Diisopropylamine
CF_3
NO_2
NO_2
N
H_7C_3
C_3H_7
$+ Pr_2N \cdot HCl$
Trifluralin

Figure 15. Gas Chromatogram of 10 Percent Technical-Grade Trifluralin

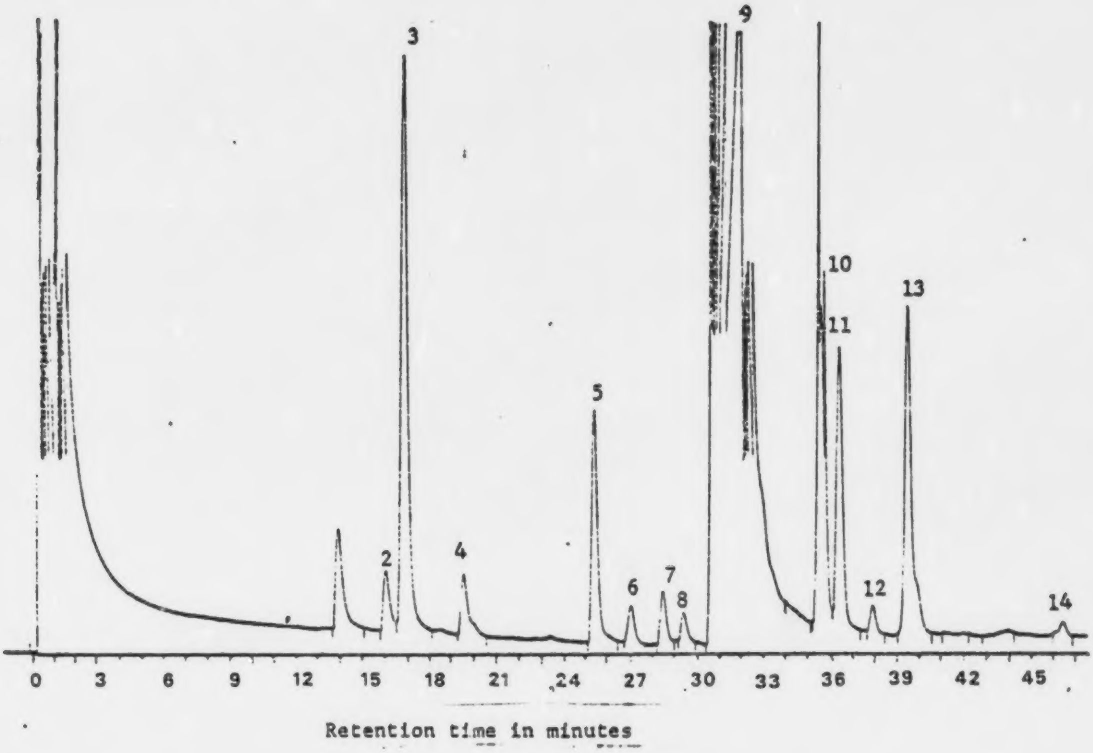


Figure 16. GC-MS of Technical-Grade Trifluralin

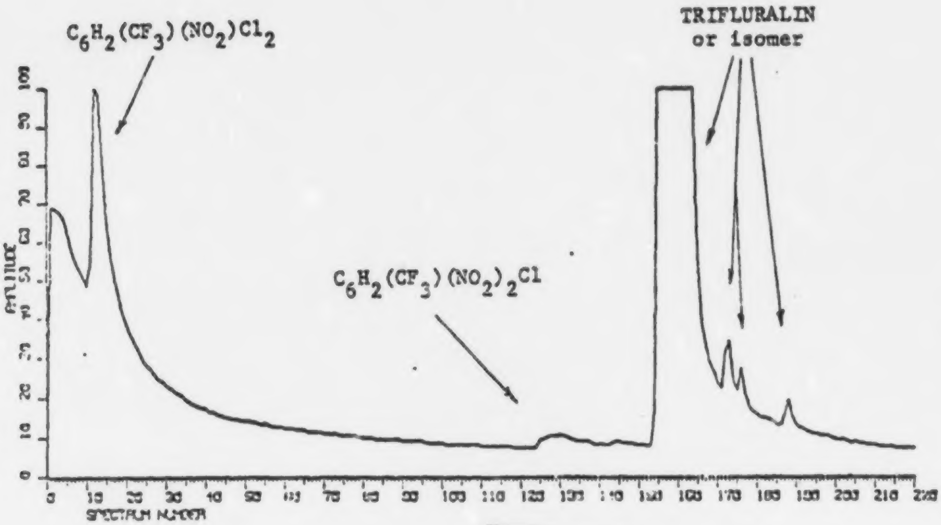


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Figure 17. Main Reactions in the Preparation of Folpet

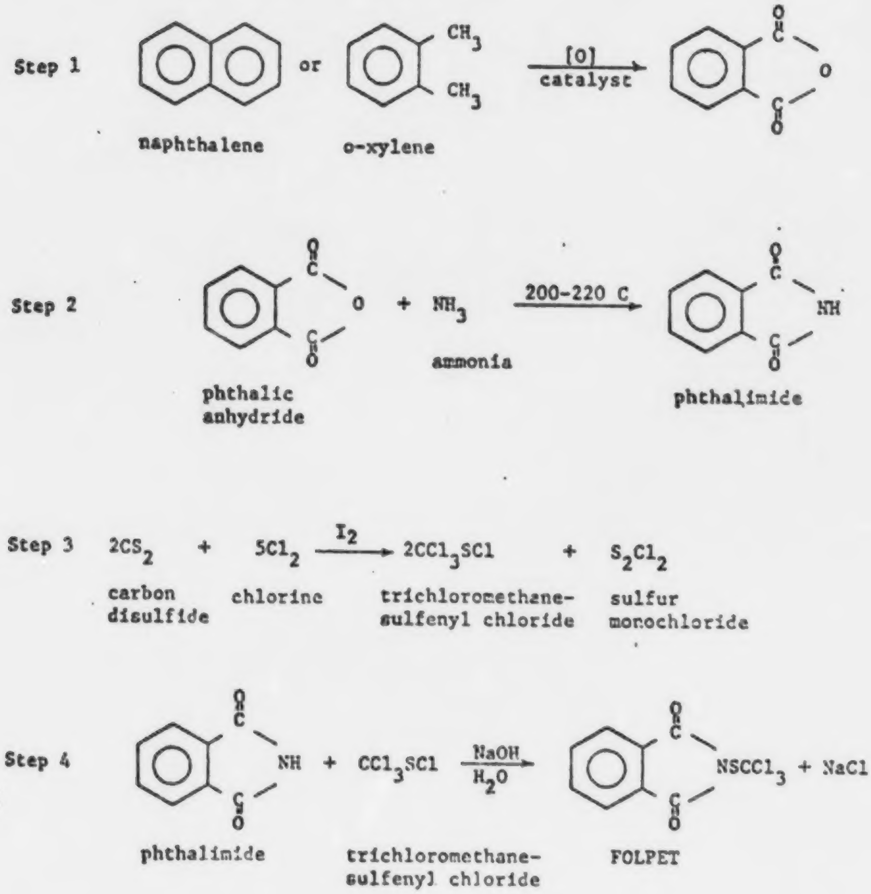

Step 1
naphthalene
or
o-xylene
CH3
CH3
[O]
catalyst
O
Step 2
phthalic anhydride
+ NH3
ammonia
200-220 C
NH
phthalimide
Step 3
2CS2 + 5Cl2
I2
2CCl3SCl + S2Cl2
carbon disulfide
chlorine
trichloromethanesulfenyl chloride
sulfur monochloride
Step 4
phthalimide
+ CCl3SCl
NaOH
H2O
NSCCl3 + NaCl
trichloromethanesulfenyl chloride
FOLPET

Figure 18. GC-MS of Technical-Grade Folpet

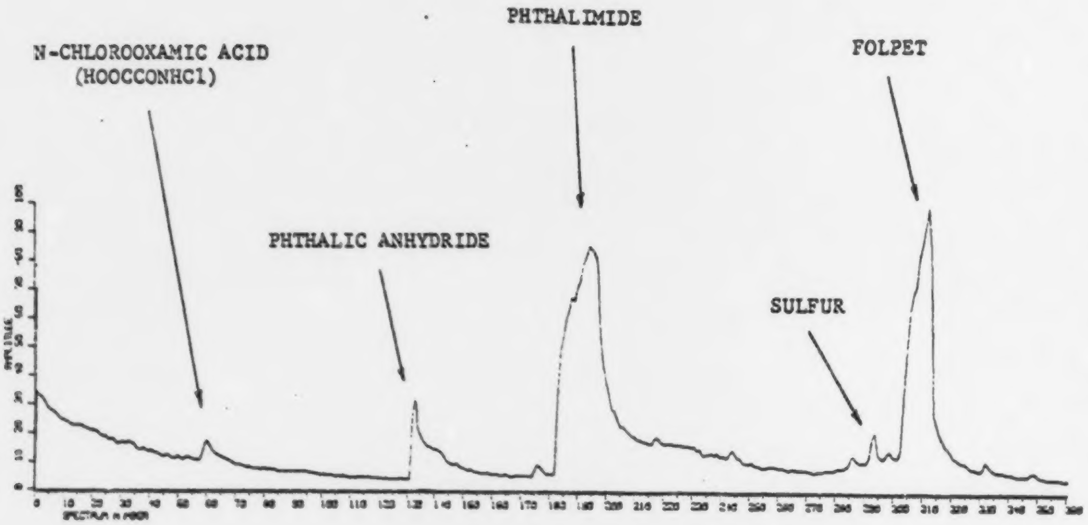


Table 3: Reactions Occurring During Production of Dursban

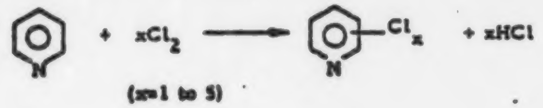
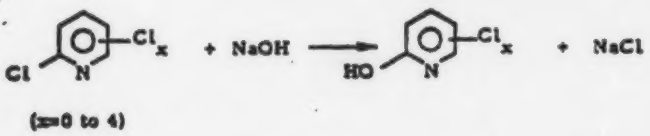
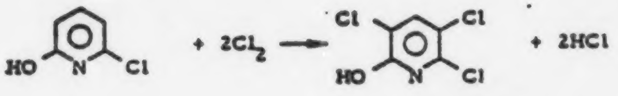
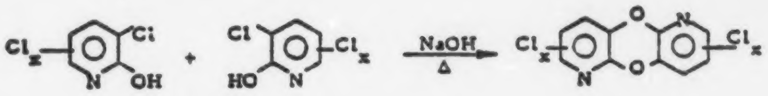
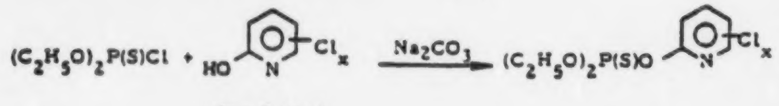
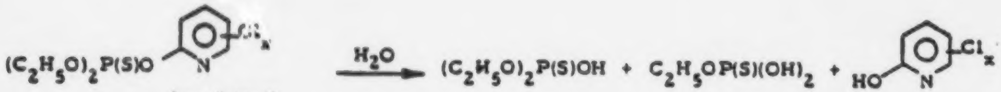
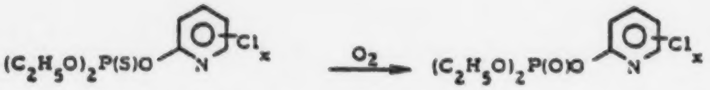
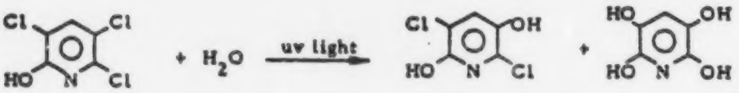
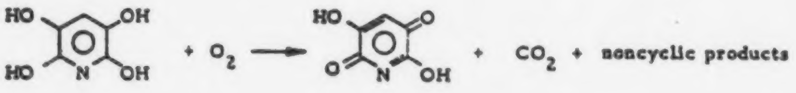
Reaction Number	Generalized Reaction[(a)]
1	
2	
3	
4	
5	$4\,C_2H_5OH + P_2S_5 \longrightarrow 2(C_2H_5O)_2P(S)SH + H_2S$
6	$(RY)_2P(Y)SH + R'OH \longrightarrow (RY)_2P(Y)SR' + H_2O$
7	$(RY)_2P(Y)SH + R'OH \longrightarrow (RY)_2P(Y)OR' + H_2S$
8	$(RY)_2P(Y)SR + R'OH \longrightarrow (RY)_2P(Y)OR' + RSH$
9	$(RY)_2P(Y)OR \xrightarrow{P_2S_5} (RY)_2P(Y)SR$
10	$(RY)_2P(Y)SH + H_2O \longrightarrow (RY)_2P(Y)OH + H_2S$
11	$(RY)_2P(S)OH \rightleftharpoons (RY)_2P(O)SH$
12	$2(RY)_2P(Y)YH \longrightarrow (RY)_2P(Y)YP(Y)(YR)_2 + H_2Y$
13	$2(RY)_2P(Y)SH \xrightarrow{[O]} (RY)_2P(Y)SSP(Y)(YR)_2$
14	$(RY)_2P(S)OR' \rightleftharpoons (RY)_2P(O)SR'$

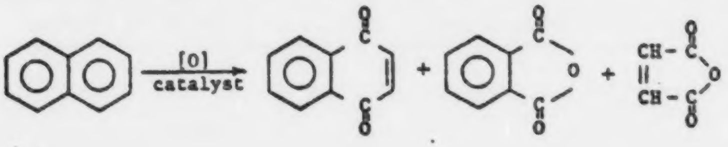
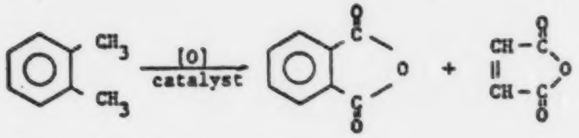
Table 3: (Continued)

Reaction Number	Generalized Reaction[(a)]
15	$(RY)_2P(S)SH \rightarrow (RY)_2PSH + S$
16	$(RY)_2PSH + R'OH \rightarrow (RY)_2POR' + H_2S$
17	$(RY)_2PSH + R'OH \rightarrow (RY)_2PSR' + H_2O$
18	$2(RY)_2P(S)SH + Cl_2 \rightarrow (RY)_2P(S)SSP(S)(YR)_2 + 2HCl$
19	$(RY)_2P(S)SH + Cl_2 \rightarrow (RY)_2P(S)Cl + S_2Cl_2$
20	 (C2H5O)2P(S)Cl + HO N Clx Na2CO3 (C2H5O)2P(S)O N Clx (x = 1 to 4)
21	 (C2H5O)2P(S)O N Clx H2O (C2H5O)2P(S)OH + C2H5OP(S)(OH)2 + HO N Clx (x = 1 to 4)
22	 (C2H5O)2P(S)O N Clx O2 (C2H5O)2P(O)O N Clx (x = 1 to 4)
23	 Cl Cl HO N Cl + H2O uv light Cl OH HO N Cl + HO OH HO N OH
24	 HO OH HO N OH + O2 HO O O N OH + CO2 + noncyclic products

---

(a) R and R' = ethyl or substituted pyridyl group
Y = oxygen or sulfur.

Table 4: Reactions Occurring During Production of Folpet

Reaction Number	Generalized Reaction[a]
1	
2	
3	$(RCO)_2O + H_2O \longrightarrow 2\ RCOOH$
4	$(RCO)_2O + NH_3 \longrightarrow RCONH_2 + RCOOH \rightleftharpoons (RCO)_2NH + H_2O$
5	$(RCO)_2NH + NH_3 \longrightarrow 2\ RCONH_2$
6	$RCOOH + NH_3 \xrightarrow{\Delta} RCONH_2 + H_2O$
7	$2CS_2 + 3Cl_2 \xrightarrow{I_2} 2CSCl_2 + S_2Cl_2$
8	$CSCl_2 + Cl_2 \longrightarrow CCl_3SCl$
9	$CSCl_2 + 3Cl_2 + 2H_2O \longrightarrow CCl_3SO_2Cl + 4HCl$
10	$2S_2Cl_2 + 2H_2O \longrightarrow 3S + 4HCl + SO_2$
11	$4CCl_3SCl \xrightarrow{Fe} S_2Cl_2 + 2CCl_4 + CCl_3SSCCl_3$
12	$2CCl_3SCl + Cl_2 \longrightarrow 2CCl_4 + S_2Cl_2$
13	$CCl_3SCl + H_2O \longrightarrow CCl_3SOH + HCl$
14	$CCl_3SOH + H_2O \longrightarrow CO_2 + S + 3HCl$
15	$CCl_3SOH + CCl_3SCl \xrightarrow{NaOH} CCl_3SOSCCl_3$
16	$2CCl_3SOSCCl_3 \longrightarrow CCl_3SO_2SCCl_3 + CCl_3SSCCl_3$
17	$CCl_3SO_2SCCl_3 + H_2O \longrightarrow CCl_3SO_3H + CCl_3SH$
18	$CCl_3SO_2Cl + H_2O \longrightarrow CCl_3SO_3H + HCl$
19	$CCl_3SCl + (RCO)_2NH \xrightarrow{NaOH} (RCO)_2NSCCl_3$
20	$CCl_3SO_2Cl + (RCO)_2NH \xrightarrow{NaOH} (RCO)_2NSO_2CCl_3$
21	$CSCl_2 + (RCO)_2NH \longrightarrow (RCO)_2NCSN(COR)_2$
22	$(RCO)_2NH + S_2Cl_2 \longrightarrow (RCO)_2NSSN(COR)_2$
23	$CCl_3SCl + RCH{=}CHR \longrightarrow R(CCl_3S)CHCHClR$
24	$RCH{=}CHR + CCl_3SO_2Cl \longrightarrow RCHClCHRCCl_3 + SO_2$
25	$(RCO)_2NSCCl_3 + H_2O \longrightarrow (RCO)_2NH + CCl_3SOH$
26	$(RCO)_2NSCCl_3 \xrightarrow{[O]} (RCO)_2NSO_2CCl_3$
27	$(RCO)_2NH + 2CCl_3SCl \qquad (RCO)_2NCl + CCl_3SSCCl_3 + HCl$
28	$RCONH_2 + 2CCl_3SCl \qquad RCONHCl + CCl_3SSCCl_3 + HCl$

a. R = alkyl or aryl group.

Table 5: Possible Contaminants in Folpet

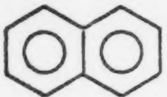
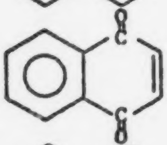
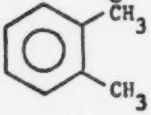
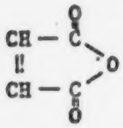
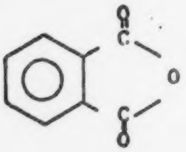
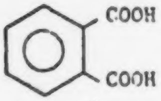
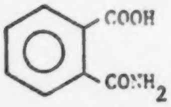
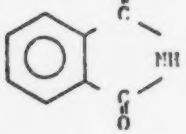
No.	Contaminant	Precursor[a]	Reaction Number[b]	Step[c]	Likelihood of Being Present in Final Product[d]
1.		SM	--	1	0
2.		1	1	1	0
3.		SM	--	1	0
4.		1 3	1 2	1 1	+
5.		1 3	1 2	1 1	++
6.	HOOCCH=CHCOOH	4	3	1 or 4	0
7.		5	3	1 or 4	+
8.		5 7	4 6	2 2	+
9.		8 26	4 25	2 St	++

Table 5. (Continued)

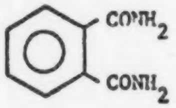
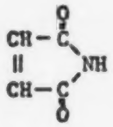
No.	Contaminant	Pre-cursor[a]	Reaction Number[b]	Step[c]	Likelihood of Being Present in Final Product[d]
10.		9 8	5 6	2 2	+
11.	$HOOCCH{=}CHCONH_2$	4 6	4 6	2 2	0
12.		11	4	2	+
13.	$H_2NOCCH{=}CHCONH_2$	12 11	5 6	2 2	0
14.	CS_2	SM	—	3	0
15.	$CSCl_2$	14	7	3	0
16.	S_2Cl_2	14 17 17	7 11 12	3 3 3	0
17.	CCl_3SCl	15	8	3	+
18.	CCl_3SO_2Cl	15	9	3	0
19.	S	16 21	10 14	3 4 or St	++
20.	CCl_3SSCCl_3	17 22	11 16	3 4 or St	+
21.	CCl_3SOH	17 26	13 25	4 4 or St	0
22.	$CCl_3SOSCCl_3$	21+17	15	4	0
23.	$CCl_3SO_2SCCl_3$	22	16	4	+
24.	CCl_3SO_3H	23 18	17 18	4 4	+

Table 5. (Continued)

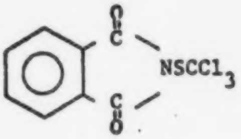
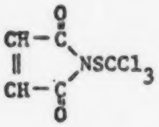
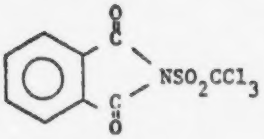
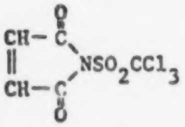
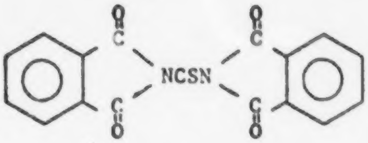
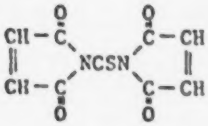
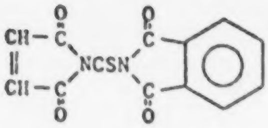
No.	Contaminant	Precursor[a]	Reaction Number[b]	Step[c]	Likelihood of Being Present in Final Product[d]
25.	CCl_3SH	23	17	4	+
26.		9+17	19	4	FOLPET
27.		12+17	19	4	++
28.		9+18 26	20 26	4 4 or St	++
29.		12+18	20	4	+
30.		9+15	21	4	+
31.		12+15	21	4	0
32.		9+12+15	21	4	0

Table 5. (Continued)

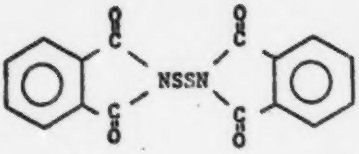
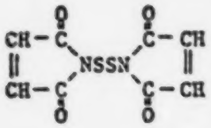
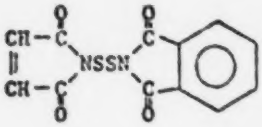
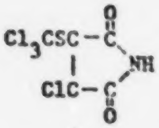
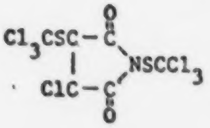
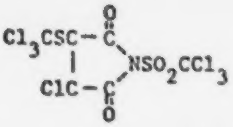
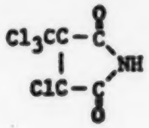
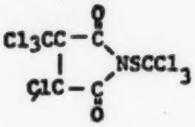
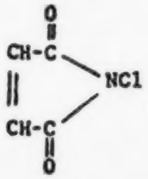
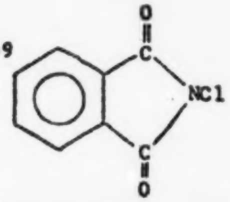
No.	Contaminant	Pre-cursor[a]	Reaction Number[b]	Step[c]	Likelihood of Being Present in Final Product[d]
33.		9+16	22	4	+
34.		12+16	22	4	0
35.		9+12+15	22	4	0
36.		12+17	23	4	0
37.		27+17 36+17	23 19	4 4	0
38.		29+17	23	4	0
39.	$HOOCCH(SCCl_3)CHClCOOH$	6+17	23	4	0
40.	$HOOCCH(SCCl_3)CHClCONH_2$	11+17	23	4	0
41.	$H_2NOCCH(SCCl_3)CHClCONH_2$	13+17	23	4	0

Table 5. (Continued)

No.	Contaminant	Precursor[a]	Reaction Number[b]	Step[c]	Likelihood of Being Present in Final Product[d]
42.		12+18	24	4	0
43.		27+18 42+17	24 19	4 4	0
44	HOOCCOOH	1 3	1 2	1 1	0
45	$HOOCCONH_2$	44	4	2	+
46	$H_2NOCCONH_2$	45	4	2	0
47	HOOCCONHCl	45	28	4	+
48		12	27	4	+
49		9	27	4	+

a. Precursor refers to the contaminant numbered in this table. SM = starting material.

b. See Table 1.

c. The step (Figure 1) during which the contaminant might be formed. St indicates possible formation during storage.

d. 0 = likely present only at levels below 1 ppm.
 + = likely present at levels above 1 ppm but below 100 ppm.
 ++ = likely present at levels above 100 ppm.

DR. LEWIS: Scott, you pointed out that you probably missed a lot of things that would not go through the gas chromatograph, but a lot of the compounds you showed, from the standpoint of polarity at least, look like the kinds of things that you wouldn't expect to go through a gas chromatograph. What sort of columns did you use, and do you have any feel as to what kind of efficiency the columns you used might have had for passing some of the polar components?

DR. WARNER: This was all done on a packed column, glass columns with on-column injection, two millimeter I.D. packed with three percent OV-17. So, it's about as nonpolar a column as you can get to try to avoid holdup of more polar materials. It's true that a lot of these materials are quite polar. That's the reason why we need to go to as high a concentration as possible, to overcome some of the absorption effects of the column.

DR. ZWEIG: Dr. Warner, I want to commend you. This is a very interesting piece of work. I have a few comments and a very few questions. Number one, I believe, and Dr. Ross might correct me, that you made a statement in your opening remarks that commercial 2,4,5-T contains less than one part per million of the dioxin. I believe it's less than a tenth of a part per million. Am I right, Dr. Ross?

DR. ROSS: Yes, you're right.

DR. WARNER: I agree.

DR. ZWEIG: Let me first make another comment. Dr. Fukuto, who unfortunately cannot be here tomorrow, actually is presenting some very interesting work which follows exactly your line of work. He shows that some of these trialkylphosphorothioates and dithioates which have been found, for example, in malathion and phenthoate, actually are synergistic and potentiate the toxicity of the parent compound. Therefore, several preparations of malathion, which in itself has an LD_{50} for rats of something like 2500 mg/kg in the presence of very small quantities of these trialkyl phosphates and phosphorothioates, the anticholinesterase compounds which you have actually found, are synergized to give an LD_{50} of a thousand or 500. So I think your observations are correct and very interesting.

The problem is not only to find out the toxicity of the impurities, but also the synergistic and potentiating effect.

PCNB is made by different manufacturers and I don't know which particular sample you got, but it is known that certain manufacturer's samples do contain a sizable quantity of hexachlorobenzene, and indeed you did find some in your particular sample. Is that correct?

DR. WARNER: Yes.

DR. ZWEIG: My last point is, technical-grade methyl parathion is sold as 80 percent material. Now, there ought to be 20 percent of a lot of impurities, and I know part of it may be xylene straight solvent, but you should have found a lot of impurities. Would you speculate on what those might be?

DR. WARNER: They're the aromatic solvent and are in the solvent front. We didn't attempt to identify those.

COMMENT: This is not a technical comment on your presentation, but just for information purposes, the FAO-WHO joint meeting has examined PCNB and it's been under review, and the existence of the hexachlorobenzene impurity, for example, that was just mentioned, is one of the more serious problems in the residues in food.

A lot of the HCB that probably shows up in certain European cheeses and dairy products is suspected to come from these sources. So, there is an obvious correlating problem here with these impurities. It's recognized in this case that it could be as much as a 4 percent impurity in some grade of PCNB. There are efforts to try to pass international regulatory guidelines to limit this to some much lower value, hopefully. So there are some efforts being made where these impurity problems are recognized.

DR. WARNER: The hexachlorobenzene that we found was under one percent.

DR. HANSON: On the last paper, I think at the end, he suggested that there may be a better method of going at it, and I know when I attended the Denver meeting, I was little chagrined at the thought that you were going to start to look for impurities in these types of products at a level down among the ten parts per million, because we have invested lots of money into one of our compounds, something about five man years within the five years looking only at levels down to 0.05 percent.

For the risk involved and the benefits, there's got to be a better way of doing it, and I think he did suggest that toxicity work, acute studies, possibly other simple type mechanisms have got to be a better way than doing it this way. Somebody's got a lifetime project for a hundred guys here. I'm not blaming Battelle -- that must be nice for them.

But, there's got to be a way other than analytical to get at this question, and I think, analytically, it's a nightmare. It's an absolute nightmare, and who is to say that the hundred parts per million is where you stop? The thrust of the whole thing is what it does toxicologically, and being a chemist and not a toxicologist, I can't comment on that, but I will. I think that some sort of screening work, to get at the answers to this, is the better way. And, of course, we do this type of screening work in that technical products are used for most of the acute battery and most of the chronic battery of tests.

Now, granted some of the metabolism work is done on pure compounds, because you can see what kind of mess you'd get into if you're trying to do metabolism on a hundred compounds at once. So, I don't mean to say that the problem is all solved before we start -- I'm just saying that we do a lot of work that says that at least the product, as we know it, toxicologically is acceptable or not acceptable. Now, I'm not talking about bioaccumulation of trace elements and everything.

But, I think we have to look at the risk benefit of the whole thing, and decide whether we can afford to go this way. Because, I don't frankly think we can. I think we're going to bog down.

QUESTION: I'd like to comment on your statement. I think, in the original work scope to this project there was to be a theoretical study of the candidate compound and to postulate or predict as many by-products that one could conceive within the reaction pot, most of which could be exonerated just through the likelihood of forming. Secondly, I thought in this theoretical part there should have been both a toxicologist as well as a teratologist working with your theoretical chemist. Should they find a potential impurity, an impurity which might be a threat toxicologically, they should decide at which levels by a quick scanning mechanism which is Part II.

And then the in-depth study that's Part III is to come. Now, I see that you did a quick GC scan, and also you did the GCMS scan. Now, have you actually done an in-depth study, even though you put the many products that could conceivably be formed in the preparation of parathion itself? Have you actually done an in-depth study of all of those? It looks very complex with the five slides that you showed of the by-products, but yet, how many products have you actually identified and really looked for in the technical compound? In what levels?

Certainly we can't go to every candidate compound and look for impurities at 0.01 part per million -- I think it was from 0.1 to 0.01 in the original proposal. I think that's been changed somewhat since the original proposal work scope came out. Most of those compounds you can exonerate initially through the theoretical study. Can you not? Would you like to comment on that?

DR. WARNER: Yes. That's true. Most of them you can exonerate, and we did not go into a more in-depth study than what I showed.

We did not have a toxicologist look them over and decide which ones can be exonerated and which ones can't. Now, I'm sure, just off the top of your head, you can decide that some of them look like they might be pretty bad species, like some of the analogs of dibenzodioxins, for example, the dipyridodioxins.

It could be a bad actor at 10 ppm or even 1 ppm. There's nothing else on our lists that might be as bad as a tetrachlorodibenzodioxin but with the dipyridodioxin we might be in the same ball park. It might require a major effort to prove that it's not present at a toxic level.

SENSORY CHEMICAL PESTICIDE WARNING SYSTEM*

Dr. Donald E. Johnson**

Restrictions on the use of persistent organochlorine insecticides have led to increased use of organophosphate and carbamate insecticides as substitutes. Many of these substitutes are highly toxic substances, manifesting their toxicity through inhibition of cholinesterase which permits the accumulation of acetylcholine to reach toxic levels. Thus, the danger in the use of certain organophosphate and carbamate insecticides is inherent. Conditions for their safe use must be determined, and rules which assure that safe conditions prevail during use must be implemented.

Although nearly all of the cases of severe poisoning or death have been among persons in the mixing, loading, or application phases of the use of these insecticides, more than 600 workers in California alone have suffered illness to some degree from organophosphate insecticides during the years 1949-1973, according to Spear et al. (1). Namba and co-workers (2) have given a higher estimate, the figure being 950 cases of poisoning due to organophosphates in the period 1957-1962. Bogden and associates (3) determined the cholinesterase activity in a sample of migrant workers in New Jersey and found that approximately one-third had activities below the lower limit of normal. Recognition of the danger of premature reentry has been given by many others over the past 25 years, notably Quinby and Lemmon (4) and Quinby et al. (5). Various Government agencies, especially EPA, have emphasized the seriousness of the threat and have taken action to reduce it.

Danger in the use of organophosphates and carbamates might be reduced substantially if a warning system were in effect in a treated area during the time that the insecticide residue remains at a health-threatening level. The purpose of the work undertaken was to develop a warning system based on the incorporation, or simultaneous but separate application, with the insecticide spray of chemical agents which were odoriferous or visible. Ideally, these agents would have volatility characteristics

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such that when they were no longer detectable, either by smell or sight, then the level of residual insecticide would be low enough to permit safe reentry. Other desirable characteristics of the agents would be low toxicity and low cost.

The three insecticides involved in this program were methyl parathion, carbofuran, and azinphos-methyl. These were chosen because they are in wide use, are representative of their chemical types, and have vapor pressures which are spread more than one order of magnitude.

Methods

Literature Search

The principal objectives of the literature search were to identify odoriferous analogs of the insecticides of interest, other compounds with outstanding odor properties (e.g., unusually bad or good odors, low odor thresholds, long-lasting), and compounds which fluoresce, phosphoresce, or change colors.

Computerized searches for these compounds were made through the following facilities: CHEMCON; DOD Technical Searching Facility; NASA Scientific and Technical Information Facility; and EPA Abstract Search Center at Research Triangle Park. However, extensive manual searching of books, reference works, and *Chemical Abstracts* was the major search effort. Additional smaller searches were required periodically in the program for technical guidance and evaluation.

During the search period, certain facts and theoretical aspects of the problem had to be considered. Items receiving consideration are mentioned in the following paragraphs.

Volatility information on the three insecticides was of prime importance. This information is given in Table 1.

Table 1: Volatility Information on Three Insecticides

Insecticide	Vapor Pressure, 20°, mm Hg	Concentration in Saturated Vapor, 20°C, ppb	Boiling Point, 760 mm Hg, °C
Methyl parathion	0.97 x 10^{-5} (6)	12.8	366**
Carbofuran	0.52 x 10^{-5}*	10	419**
Azinphos-methyl	2.2 x 10^{-7}(6)	0.03	500**

*Extrapolated value based on vapor pressure data at higher temperatures supplied by manufacturer.

**Extrapolated values based on information from reference (6) and manufacturer. Decomposition probably occurs before these temperatures are reached.

Requirements for ideal odor agents were envisioned to be as follows:

- Vapor pressures should be close to those of the insecticides;
- Molecular weights should not exceed 300 and, preferably, should be below 250. This requirement is based on the observation by Stoll (7) that no odorous compound is known with a molecular weight above 300 and that the limit of perception, for many people, begins around 250;
- Odor perception thresholds should be below the saturated vapor concentrations shown in Table 1 for the three insecticides. Preferably, the thresholds should be much below these values since the atmosphere sniffed in actual field conditions would never be saturated;
- Chemical characteristics should be similar to the three insecticides since the disappearance time of an insecticide is thought to be the sum of losses from such factors as evaporation, hydrolysis, water solubility, photolysis, absorption, microorganism action, oxidation, and isomerization. Evaporation from surface deposits is thought to be a major route of disappearance for many insecticides (8);
- The agents should be relatively nontoxic originally and when they degrade;
- Odors of the agents should evoke a sharp human response;
- The agents should not leave unacceptable residues.

Laboratory Disappearance Tests

The first experimental effort was to determine the disappearance times of the three insecticides of concern, potential odor agents, and potential visual agents. The objective was to match the disappearance times of the three insecticides with odor agents and visual agents.

This work encountered the problem of uneven distribution of the substance under study. A dilute solution of a substance in a volatile solvent does not deposit the substance uniformly if permitted to evaporate unmolested on a flat surface. Uneven distribution leads to unequal disappearance times for the areas of relatively heavy and light deposits. Miniature spray devices were found difficult to control when spraying the small areas needed in the studies, and the stray spray droplets required safety precautions which were onerous.

Eventually, a system was developed which gave a fairly uniform distribution of insecticides and sensory agents on glass plates which were then exposed to controlled conditions in the laboratory. A 4-inch frosted glass square was rotated on a turntable at 175 rpm while a selected volume of agent or insecticide in solution in toluene was applied to the center of the plate. Perfect reproduction of area covered and perfect uniformity of the deposit were not achieved, but the system gave fair-to-good reproducibility and was very useful. When the amount of solution deposited is 50 microliters, the area covered is approximately 30 cm^2. When all factors involved except concentration are constant, then the amount of substance deposited per cm^2 becomes dependent upon the concentration of substance in the solution. Disappearance tests were conducted at a temperature of 23° C (73° F) and with an air velocity over the plates of 8 kph (5mph).

In order to follow the disappearance of the insecticides, an analytical capability of adequate sensitivity was established using a gas chromatograph equipped with a flame ionization detector.

Carbofuran was found to chromatograph satisfactorily on a 20-inch, 1/8-inch diameter SS tube packed with 10 percent UC-W98 on Chromosorb WAW-DMCS (80-100 mesh) at an oven temperature of 150° C. Other conditions are: carrier gas flow = 30 ml/min.; H_2 flow = 30 ml/min.; O_2 flow = 240 ml/min.; injection port temperature = 200° C; and detector temperature = 200° C. Methyl parathion was analyzed on the same column at the conditions above, except an oven temperature of 160° C was preferable. Azinphos-methyl was also chromatographed on this column but at temperatures of 250° C for the injection port and detector and 200° C for the oven.

In order to determine the amount of insecticide present on the glass plates at the desired intervals after application, a plate was rinsed with a stream of dichloromethane which was collected as it ran from the corner of the plate in a small glass beaker. The rinse was evaporated without heating, under a stream of nitrogen, to predesignated volume and compared with a standard solution by gas chromatographic analysis.

The odor agents were evaluated by two or more individuals sniffing the plates. The visual agents were evaluated by two or more individuals observing the plates under short wave (254 nm) ultraviolet light.

Field Tests

Outdoor disappearance tests were conducted for the three insecticides by spraying several plants of the ornamental species Euonymus japonica and exposing them to the environment in the vicinity of the laboratory. Periodically, leaves were removed for the purpose of determining the amount of residual insecticide. The plants were sprayed with emulsifiable formulations commonly used in agricultural practice. The spray was applied until the plant leaves were dripping wet; then the excess was removed by tilting the plants and gently shaking until obvious pools disappeared.

At the same time, sensory agents were evaluated by exposing them by incorporation in the spray, on glass plates, in plastic membranes, and on paper strips. The purpose of these tests was to ascertain whether or not the laboratory tests conducted earlier gave valid information and to prepare for a field test later on involving commercial crop spraying.

Methyl parathion E-4 was applied at a theoretical coverage of 5.0 $\mu g/cm^2$ (0.44 lb./acre). During the day, the plants were kept outdoors. At night they were taken into a greenhouse, since freezing at night was a possibility. The outdoor temperature extremes during the exposure periods of the tests were 4 to 24° C (40 to 76° F). Sunshine was plentiful and the relative humidity was generally low. Wind velocity varied from calm to 24 kph (15 mph). The greenhouse temperature varied from 25 to 29° C (78 to 85° F), and the relative humidity was high. Two tests were conducted, each of 3 days duration.

Guthion 2L (azinphos-methyl) and Furadan 4 Flowable (carbofuran) were applied at a theoretical coverage of 10 $\mu g/cm^2$ (0.88 lb./acre). The plants sprayed with these products were kept outside except at night and when rain threatened. During these times, the plants were placed under a shelter which consisted of a protective roof without sides. Thus, the plants were sheltered from direct rainfall but were otherwise exposed to the other atmospheric factors. Shielding from direct rainfall was decided upon so that the disappearance time experienced would probably be a maximum one under the test circumstances, rather than a minimum one which would result if a downpour of rain was found to wash the plants free of the pesticide. Even so, the plants were wetted often with condensed moisture during foggy weather and when the dew point was reached. During the 55-day test period, the following general statements about the weather apply:

- Temperature extremes were 0.5° C (33° F) and 32° C (90° F).
- Wind speeds were most often 16-40 kph (10-25 mph) with occasional gusty periods where the wind speed reached as high as 72 kph (45 mph).
- The relative humidity was usually moderate; however, extremes were experienced.
- Cloudy skies prevailed more than 50 percent of the time.

Subsequent to the above outdoor tests, a limited field test was conducted on a cotton farm near Batesville, Texas. The cotton plants, in the early blooming stage, were sprayed by airplane with methyl parathion at an intended dosage of 17 $\mu g/cm^2$ (1.5 lb./acre) and Galecron at an intended dosage of 0.95 $\mu g/cm^2$ (0.083 lb./acre). The main purpose of the test was to follow the disappearances of the methyl parathion from the cotton leaves and of visual and odor agents placed alongside of the field. Leaf samples were taken at 20 minutes, 2, 4, 8, 22.5, and 48 hours after application. Observations of the sensory agents were made at the 4, 8, 22.5, and 48-hour points.

Spraying was completed at approximately 8 a.m. At that time, the wind was blowing at an estimated 30-40 kph (18.5-25 mph). During that day and the remainder of the test period, the wind blew steadily at that velocity, or slightly higher, with frequent gusts to 56 kph (35 mph), except for the period near dawn when the velocity dropped to the 8-16 kph (5-10 mph) range. The days were sunny with only scattered light clouds. The temperature span during the test period was from 25.6 to 34.4° C (78 to 94° F). The relative humidity ranged from high in the mornings to moderately low in the afternoons. No rainfall occurred during the test period.

Prior to the spraying, a small table was erected approximately 150 meters into the field so that the table top was essentially at cotton plant height. Twelve glass plates, 5 x 5 cm^2, were fastened to the table top by placing them on strips of tape having adhesive on both sides. This was done so that the turbulent air streams from the spraying airplane could not dislodge the plates from the table top. The purpose of the plates was to collect spray for analysis and comparison with leaf samples and the sensory agents on glass plates. After the first samples were taken, the table top was removed from the supporting legs and kept in the shade of the cotton plants. It had been found in the earlier preparatory tests that exposure in the shade more closely matched the disappearance of a substance from a glass plate with the disappearance of that same substance from a plant in the sun.

At the time that the table with the glass plates was set up, 10 cotton plants surrounding the table (none closer than 3 meters, none farther away than 20 meters) were tagged with white cloth strips. This was done so that sample leaves would always be taken from the tagged plants or the ones on either side of them.

After spraying was completed, sampling consisted of removal of two of the glass plates and one canopy leaf from the windward side of each of the designated plants (or the ones on either side of them). The upper sides (exposed to the aerial spraying) of the plates were washed thoroughly with a small stream of dichloromethane (DCM), and the washings were collected in a single glass bottle for transport to the laboratory for analysis. The leaves were placed on each other, making a stack 10-high, on a polyethylene-covered board. A No. 15 laboratory cork borer was used to cut through the leaves, giving a circular sample of each leaf of about 2 cm in diameter. This sample was ejected from the borer into a glass beaker, and the process was repeated two times, with each set of leaf samples being put into a different beaker. Each set of 10 circles was washed 3 times with 15 ml of DCM, using a small stainless steel spatula to make sure that agglomerates of leaf circles were broken up and each circle contacted by the solvent. The washings from each set of 10 circles were bottled in glass bottles and transported to the laboratory for analysis.

At the laboratory, each bottle of DCM washings was reduced to the standard volume of 3 ml under a stream of nitrogen without heating, and the concentrate was analyzed by gas chromatography under conditions given above. The concentration of methyl parathion in each sample was calculated from peak height measurements on the chromatograms of the samples and a standard solution of methyl parathion present on a per-square-centimeter basis of upper leaf surface or upper plate surface. Samples of leaves taken before spraying showed no interference on the chromatograms at the points of interest.

During the hour preceding the spraying, two visual agents and three odor agents were put under test in a shaded area beside the cotton field. The visual agents were anthracene and phenanthrene. The odor agents were skatole, 2-phenylethanol, and β-phenylethylphenylacetate. The visual agents were applied to frosted glass plates only. The odorant skatole was applied to a frosted glass plate and also to paper strips. The other two odorants were applied only to paper strips. Observations were made during the test by the two men conducting the test. Attempts were made to procure disinterested observers from the population in the vicinity, but the remoteness of the field made such arrangements unworkable. As a consequence, an opinion survey was taken, at another site, of the response of five field workers to the sensory items.

Cost Analysis

The first step taken in the cost analysis was to develop concepts of packaging the warning items and methods of field use. These concepts were restricted to systems to be used with methyl parathion, since warning systems devised for this widely used insecticide seem to be most readily applicable for field use. With packaging and use concepts in mind, cost estimates were prepared for three methods of field application as being the sum of these cost factors: 1) product development, 2) manufacturing cost, 3) distribution cost, 4) installation cost, and 5) operational costs. Information on the usage and cost of methyl parathion was obtained from pertinent literature and by telephone interviews with knowledgeable persons at several universities and agricultural extension services, and incremental additional costs of the use of the warning system were estimated. Cost estimates are based on data for similar mass-produced items using financially conservative figures, i.e., benefits were given minimal values and costs were given maximum values in terms of 1975 dollars.

Results

Literature Search

The literature search did not reveal any candidate odor compounds from the same chemical families as the insecticides. Use of the modifiers "odor," "smell," or "odoriferous" in connection with names of the insecticides, or their families, always resulted in zero citations, whether the search was by computer or manual. These negative results offer the options for believing that there are no such compounds or, if such compounds exist, their odoriferous characters are not cited as key pieces of information. The insecticides of interest have appreciable odors emanating from the emulsifiable concentrates, but manufacturers' representatives are of the opinion that most of the odors are from low molecular weight, volatile compounds present as minor impurities. Hence, the odors dissipate rather rapidly from the areas treated

Table 2: Candidate Odor Agents

Compound	Mol. Wt.	Boiling Point(15)		Odor Thresh., ppb (v/v)(16)	Odor Character
		t, ° C	pr., mm Hg		
artificial musk, 1-tertbutyl-3-methyl-2,4,6-trinitrobenzene	283	m.96-7	--	0.001	like natural musk
typical synthetic macrocyclic musk, 11-oxahexadecanolide	256	m.35	--	--	like natural musk
musk ambrette	268	25	2.5×10^{-5}	--	like natural musk
musk xylene	297	25	$1. \times 10^{-5}$	--	like natural musk
musk ketone	294	25	2.4×10^{-6}	--	like natural musk
muscone-active principle in natural musk, 3-methylcyclopentadecanone	238	328 130	760 0.5	--	natural musk
civetone, 9-cycloheptadecen-1-one	250	342 159	742 2	-- --	disgustingly obnoxious, becoming pleasant in extreme dilutions
2-hexyl-3-methoxypyrazine	194	--	--	0.001(17)	like fresh bell pepper
2-isopropyl-3-methoxypyrazine	152	--	--	0.002	"
2-isobutyl-3-methoxypyrazine	166	--	--	0.002	"
2-propyl-3-methoxypyrazine	152	--	--	0.006	"
alpha-ionone, beta-ionone	192	127 81 250	12 1 760	0.013	like cedar wood in strong dilutions; like violets in extreme dilutions.
vanillin	152	285 170	atm. 15	0.016	like vanilla
ethyl vanillin	166	.25	1.7×10^{-4}	--	like vanilla
deca-trans, trans-2,4-dienal	152	--	--	0.070(18)	like fried chicken
2-methoxynaphthalene	158	272	atm.	0.12	like old nerolin

Table 2: Candidate Odor Agents (cont.)

Compound	Mol. Wt.	Boiling Point (15)		Odor Thresh., ppb (v/v)(16)	Odor Character
		t, °C	pr., mm Hg		
coumarin	146	298 139	atm. 5	0.28	like vanilla
methyl coumarin	161	305	760	--	like vanilla
methyl anthranilate	151	135	15	0.65	like concord grapes
1,7,7-trimethylbicyclo[4.4.0]-decan-3-one	195	87	0.001	--	can be used in perfumes
1,7,7-trimethylbicyclo[4.4.0]-decan-3-formate	224	85	0.01	--	"
" -acetate	238	90	0.01	--	"
" -propionate	252	100	0.02	--	"
" -butyrate	266	115	0.05	--	"
" -acrylate	250	86	0.01	--	"
alpha-(2-hydroxycyclohexylmethyl) butyrolactone	196	203	3	--	like peppermint
alpha-(4-hydroxycyclohexylmethyl) butyrolactone	196	203	3	--	like peppermint
1-(phenylethoxy)adamantane	256	176	1	--	can be used in perfumes
gamma-decalactone	170	281	atm.	--	like peach
gamma-undecalactone	184	286	atm.	--	like peach
gamma-dodecalactone	198	170	11	--	like peach
benzophenone	182	305	760	--	--
phenylethyl phenylacetate	240	324	760	--	rose-hyacinth

Table 2: Candidate Odor Agents (cont.)

Compound	Mol. Wt.	Boiling Point[15] t, °C	pr., mm Hg	Odor Thresh., ppb (v/v)[16]	Odor Character
benzyl cinnamate	238	350	760	--	sweet odor of balsam
hexyl cinnamic aldehyde	216	305	760	--	--
benzyl benzoate	212	323	760	--	faint, pleasant, aromatic
benzyl salicylate	228	368	760	--	pleasant
o-bromophenol	170	194	760	0.001	unpleasant
alpha and beta-santalol	220	302	760	--	like sandalwood

Note: Compounds for which no odor threshold data are presently available were included in the above list on the basis of actual or estimated high boiling points and indicated odorous character.

with the insecticide sprays. Methyl parathion does release methyl mercaptan very slowly by hydrolysis, but a satisfactory warning odor level is not reached under use conditions.

At this point in the program, with the theoretically most promising chemical categories having been eliminated, attention was turned toward compiling a list of odoriferous compounds with emphasis on those boiling above 275° C and having, or estimated as having, low odor thresholds. This list is given in Table 2. While a number of very odoriferous substances are listed in Table 2, only some members of the musk family, benzyl salicylate, and benzyl cinnamate have vapor pressures approaching those of the three insecticides. On this basis alone, these compounds appeared to be the best odor candidates in this program. However, this turned out not to be the case for reasons given below.

Table 3 lists fluorescent compounds having boiling points of interest. Several compounds from this list were selected for disappearance testing, as mentioned below.

Laboratory Disappearance Tests

Working with emulsifiable concentrates commonly used, disappearance times were determined for methyl parathion E-4, Furadan 4 Flowable (carbofuran), and Guthion 2L (azinphos-methyl). Two levels of deposit of each insecticide were applied in this work, 10 $\mu g/cm^2$ (0.88 lb./acre) and 5 $\mu g/cm^2$ (0.44 lb./acre), simulating typical applications in the field. At both levels of application, only about 3 percent of the methyl parathion remained on the plate after 24 hours had passed. The high level of Furadan showed about 20 percent remaining after 15 days and the medium level about 3 percent remaining after 12 days. The high level of Guthion showed about 60 percent remaining after 30 days, with this dropping to around 12 percent after 60 days. The medium level of Guthion was not followed beyond the 28-day mark, at which point about 20 percent remained. The results are shown graphically in Figures 1, 2, and 3.

Fluorescing agents were usually screened first at application levels of 10, 1.0, and 0.1 $\mu g/cm^2$. Then the application level was adjusted upward or downward until

Table 3: Candidate Visual Agents

Compound	Boiling Point, °C
trans-stilbene	305 (720 mm Hg)
anthracene	340
phenanthrene	340
acridine	345-6
alpha-benzylnaphthalene	350
para-benzylnaphthalene	350
1,4-diphenylbutadiene	350 (720 mm Hg)
4-methoxybenzophenone	354-5
carbazole	355
triphenylmethane	358-9
2,5-diphenyloxazole	360
1,1,2,2-tetraphenylethane	360
2-hydroxybenzothiazole	360
diphenylurethane	360
triphenyl phosphite	360
quinazolinone	360
benzimidazole	360
1,2-benzophenazine	360
1,1'-binaphthyl	360+
1,3,5-trinitronaphthalene	364 (explodes)
1-naphthyl-2-tolyl ketone	365
m-terphenyl	365
diphenylene disulfide	366
phenothiazine	371
thioxanthone	372
fluoranthene	375
p-terphenyl	376
anthraquinone	377
diphenylsulfone	379

Table 3: Candidate Visual Agents (cont.)

Compound	Boiling Point, °C
melene	380
triphenylmethanol	380
retene	390
pyrene	393
N-benzylsuccinimide	395
4,4'-dibromobenzophenone	395
4,4'-ditolysulfone	405 (714 mm Hg)
1,2-benzofluorene	413
9-phenylanthracene	417
o,o'-quaterphenyl	420
tetraphenylethylene	420
triphenylene	425
9,10-benzophenanthrene	425
1,2-dihydroxyanthraquinone	430
tetraphenylmethane	431
1,2,-benzanthracene	435
1,8-dinitronaphthalene	445 (decomposes)
chrysene	448
2,2-binaphthyl	452
1,3,5-triphenylbenzene	459 (717 mm Hg)
1,2,6-trihydroxyanthraquinone	459
1,2,7-trihydroxyanthraquinone	462
11,12-benzofluoranthene	480
picene	519

Figure 1. Disappearance of methyl parathion from glass plates

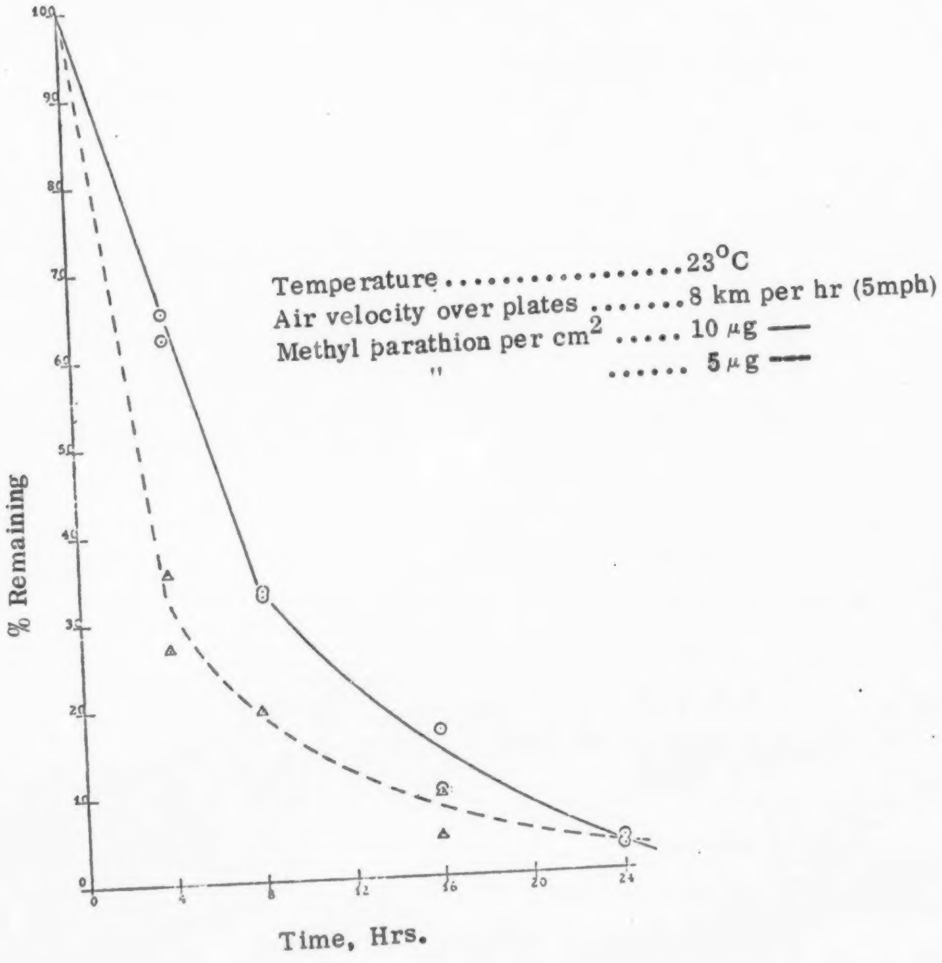


Figure 2. Disappearance of Furadan from glass plates

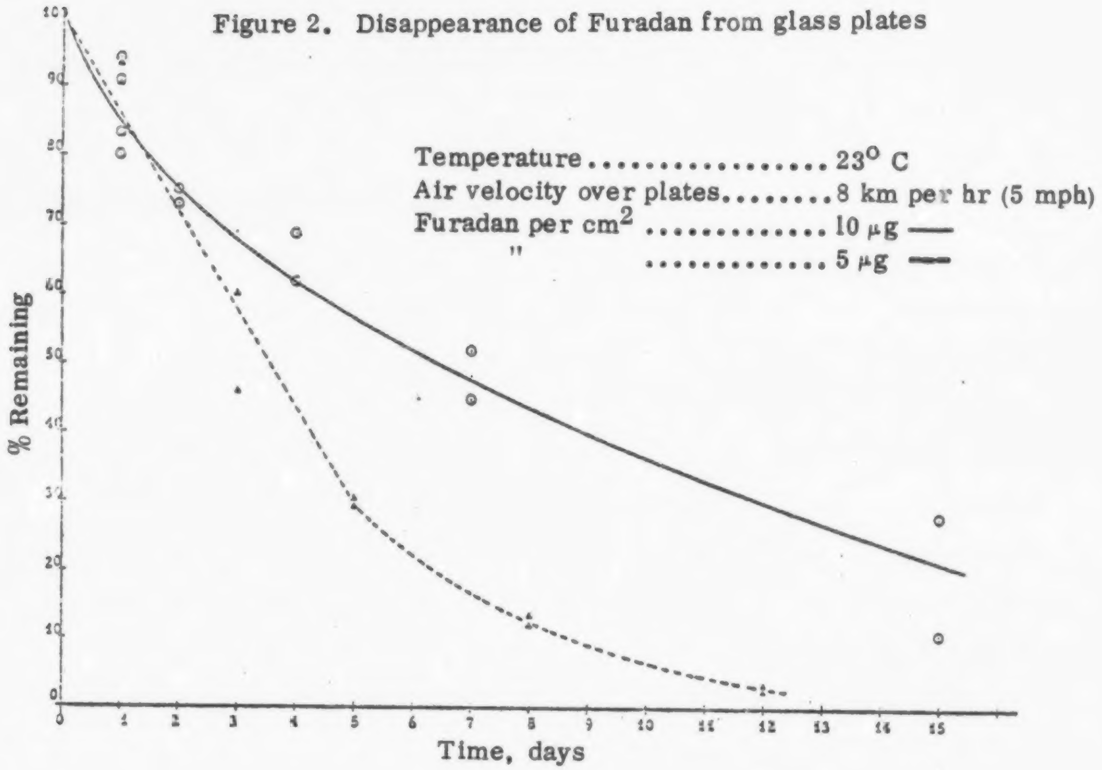
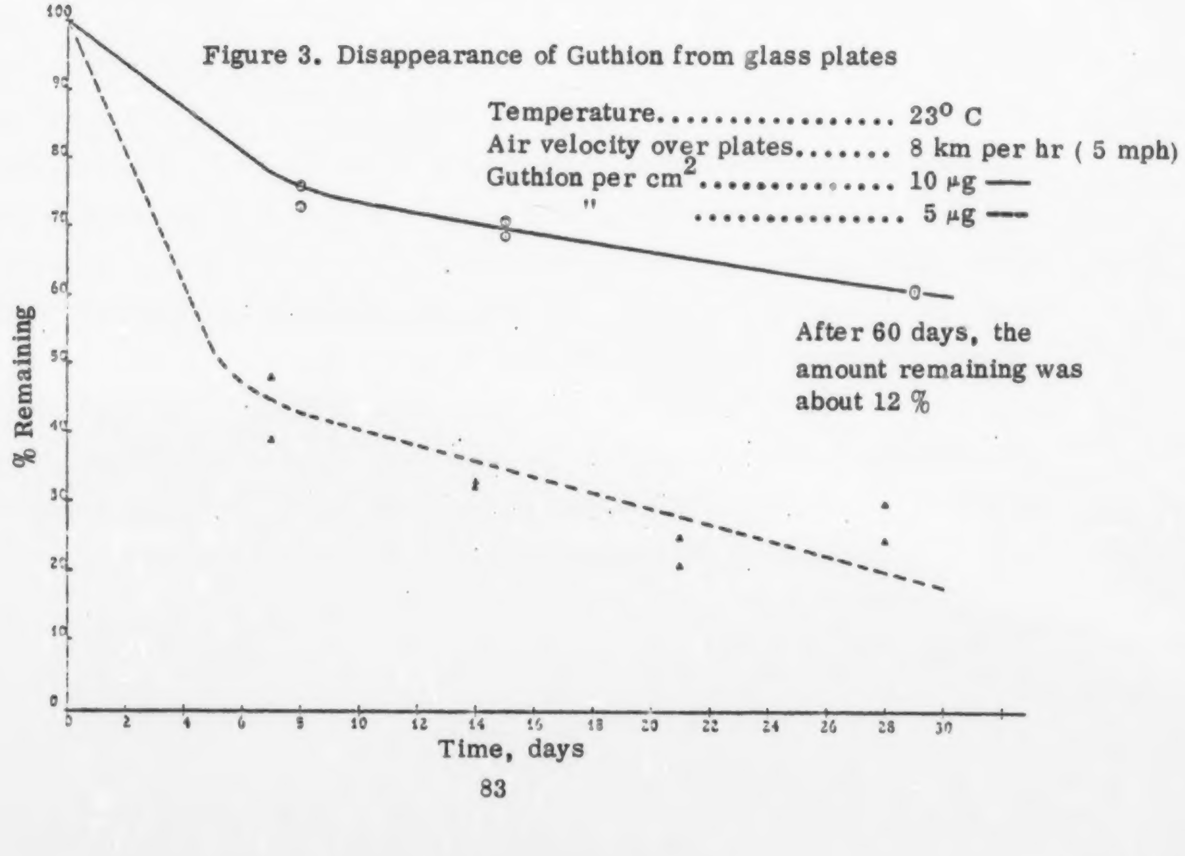


Figure 3. Disappearance of Guthion from glass plates



After 60 days, the amount remaining was about 12 %

Table 4: Typical Data for the Disappearance of Fluorescing Agents from Glass Plates

Fluorescing Agent and Level of Application, μg/cm^2		Visual Fluorescing Strength* Hours 0	16	20	24	30	40	44	48
Anthracene	5	S	S	S	M	M	W	W	O
Anthracene	3	S	S	S	S	M	W	O	
Anthracene	2	S	W	O					
Phenanthrene	50	S	S	S	S	W	W	O	
Phenanthrene	40	S	W	W	O				
Phenanthrene	20	S	O						
Fluoranthene	5	S	S	S	M	M	W	O	
Fluoranthene	3	S	M	W	O				

---

*S = strong; M = moderate; W = weak; O = none

disappearance times that were thought to reasonably match or bracket the disappearance time of an insecticide were attained. Table 4 shows some typical disappearance data acquired while trying to match the laboratory disappearance time of methyl parathion which was 24 hours or slightly more. Disappearance data were acquired on the following compounds in addition to the three shown in Table 4: carbazole, triphenylmethane, trans-stilbene, 6-methylcoumarin, 2,5-diphenyloxazole, chrysene, pyrene, o-quaterphenyl, and p-terphenyl. Disappearance times in excess of 25 days were attained with carbazole, one of the outstanding long-term fluorescent compounds. Chrysene, another long-term agent, was eliminated from the study because it is weakly carcinogenic (10). These tests indicated that the laboratory disappearance times of all three of the insecticides could be matched under laboratory conditions and that anthracene and phenanthrene were as good as or better than the other compounds for use with methyl parathion.

In the course of the screening of visual agents, the fluorescent compounds were sometimes mixed with insecticides. Occasionally, partial or complete quenching of the fluorescence resulted. The possibility of putting an insecticide over a fluorescing substance, which could then not be seen until the insecticide disappeared, was intriguing as a concept for a sensory warning system. However, this phenomenon was found to be erratic and uncontrollable in the limited tests which were conducted to explore it.

Candidate odor agents were screened initially as were the visual agents. At first, they were applied at a coverage of 10 $\mu g/cm^2$. However, none of the odorants tested gave much residual odor after 24 hours had passed. By increasing the amount applied by a factor of 2 or more, residence times of 1 to 3 days could be achieved with compounds such as the musks, vanillin, and skatole. The simultaneous application of fixatives, such as mineral oil, were not of any substantial help. However, residence times could be increased drastically by mixing the odorant in a solution of a polymer, then casting the solution in thin films. The polymers used in this application were Ethocel (Dow ethylcellulose - 48 to 49.5 percent ethoxy content - 10 cps) and Vinylite VYHH (Union Carbide copolymer of 86 percent vinyl chloride and 14 percent vinyl acetate). This was the technique devised by which the long residence times of carbofuran and azinphos-methyl could possibly be matched.

Screening tests were conducted on the compounds listed in Table 5. The candidates which had boiling points nearest those of the insecticides (the artificial musks, benzyl salicylate, and benzyl cinnamate) were judged unacceptable because of their mild odor character. Strongly odoriferous compounds, when applied by themselves, did not have adequate residence times. The best position to take appeared to be a compromise position in which the desired high boiling point characteristic is traded off in favor of increased odor intensity. However, in order to use such substances, which are quite volatile relative to the pesticides carbofuran and azinphos-methyl, the evaporation

Table 5: Candidate Odor Agents Subjected To Screening Tests

Benzyl cinnamate
Benzyl salicylate
beta-Phenylethylphenylacetate
Coumarin
Ethyl vanillin
2-Methoxynaphthalene
Santalol
Vanillin
Methyl anthranilate
Ethyl anthranilate
Ethyl salicylate
Eugenol--USP--prime
Sandela
Musk ketone
Musk ambrette
Musk xylol
Hexadecanolide
Heptaldehyde
2-Phenylethanol
Pentadecanolide
Anisyl acetate
Terpineol
Laurine
Jasmonyl
Methyl eugenol
Geraniol
Phenoxyethyl isobutyrate
Anisyl alcohol
Acetanisole
Galaxolide (musk)
6-Methylcoumarin
gamma-Dodecalactone
Skatole
alpha-Chloroacetophenone
alpha-Bromoacetophenone
Menthol
Dimethyl sulfide
Several proprietary perfume oils and odor masking agents

had to be retarded by the polymers mentioned above. In the case of methyl parathion, its disappearance time could be matched by adjusting the quantities of the odor agent applied to the glass plates or to paper strips. Matching could also be achieved by incorporating the odor agent in the methyl parathion prior to spraying.

Through these tests, good matches in disappearance times could be achieved with respect to methyl parathion, and matching the disappearance times of carbofuran and azinphos-methyl appeared possible. The odorants chosen for the field work were skatole, β-phenylethylphenylacetate, 2-phenylethanol, eugenol, and α-bromoacetophenone.

Field Tests

The disappearance curve of methyl parathion from the ornamental species *Euonymus japonica* is shown in Figure 4. The time required for more than 90 percent to be gone is about three times that found in the laboratory (see Figure 1). This may be the result of the much colder average temperature to which the plants were exposed, since the test was conducted in mid-winter, as compared to the laboratory tests. This increase in disappearance time was matched by increased times for the sensory agents exposed on and beside the plants. In this test, anthracene, phenanthrene, and skatole functioned well as sensory agents. Eugenol and α-bromoacetophenone were also tested as odorant sensory agents at this time, but they were judged to be inferior to skatole.

Disappearance curves for methyl parathion from the cotton leaves and glass plates during the Batesville test are given in Figure 5. The three values for leaf samples at each sampling time were averaged before that disappearance curve was constructed. The methyl parathion disappeared a bit more rapidly from the cotton leaves than from the glass plates. At 24 and 48 hours, approximately 3 percent and 1 percent, respectively, remained on the leaves, while about 14 percent and 4 percent remained on the plates at those time points.

The observations made on the sensory items by the two men conducting the test are given in Table 6 in terms of whether or not a definite visual or odor response was noted and the intensity of that response or signal. It may be noted that

Figure 4. The disappearance of methyl parathion from Euonymus plants

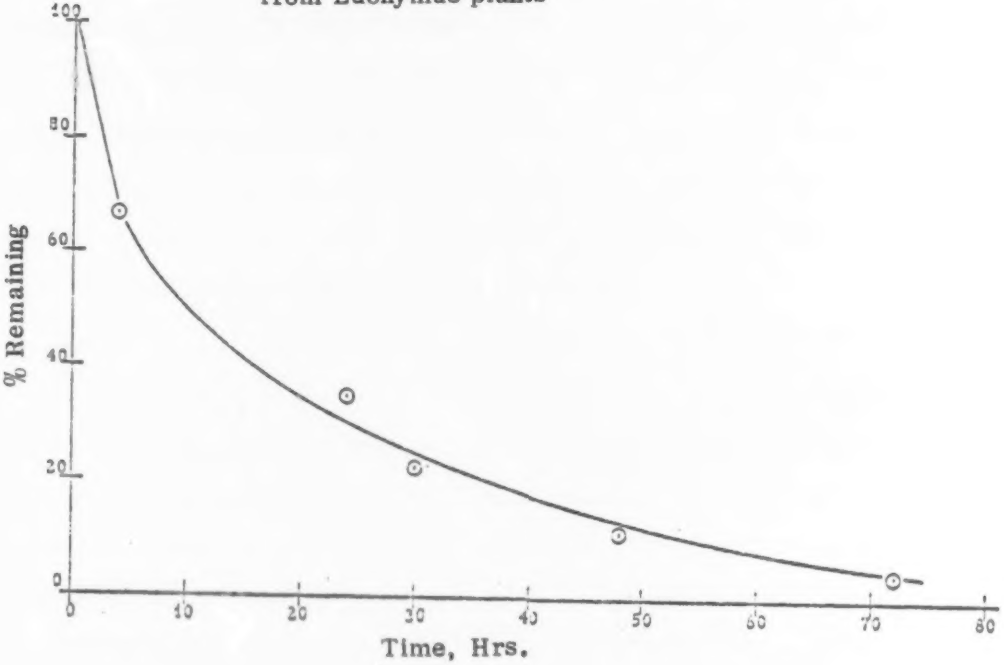


Figure 5. The disappearance of methyl parathion from cotton leaves and glass plates

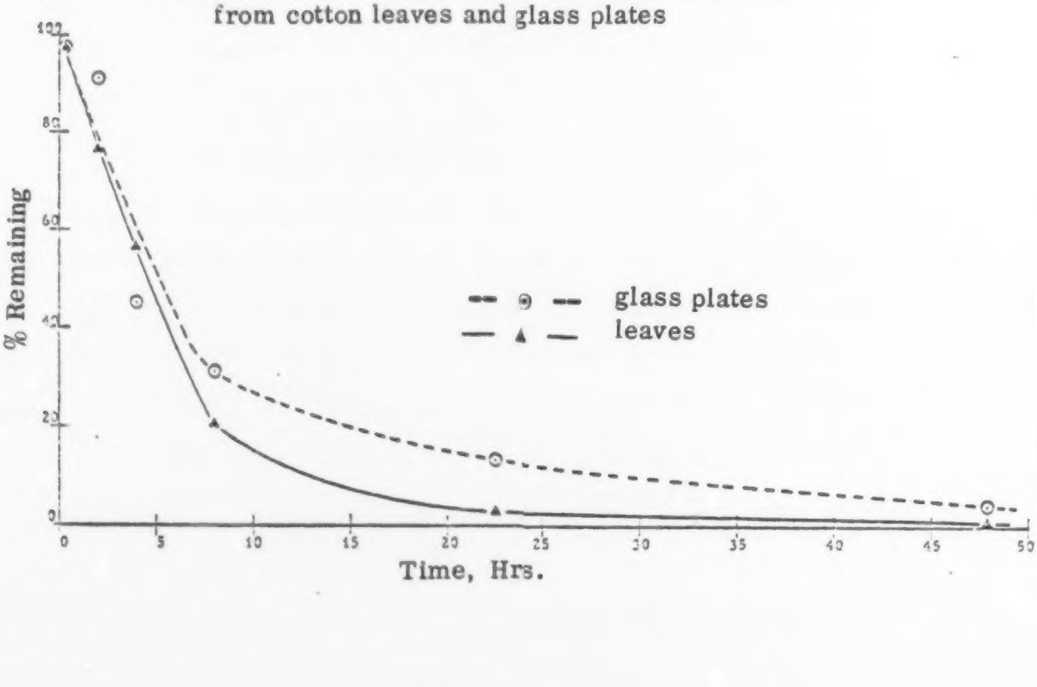


Table 6: Observations Made on Sensory Items During Batesville Field Test

		Time, Hours				
Compound	Conc. $\mu g/cm^2$	0	4	8	22.5	48
Anthracene	3	S	W	E		
	5	S	S	W	E	
	7.5	S	S	S	I_1	E
	10	S	S	S	I_2	E
Phenanthrene	40	S	W	E		
	50	S	W	E		
	60	S	W	E		
Skatole (on plate)	40	S	S	S/W	W/E	E
	g/l					
Skatole (on paper)	6	S	S/W	W/E	E	
	9	S	S	S/W	E	
	12	S	S	S	W/E	E
	24	S	S	S	M	VW
2-Phenylethanol	1	S	S	S/W	M	VW
	3	S	S	S	M	M
	6	S	S	S	M	M
	9	S	S	S	M	M
β-Phenylethyl-phenylacetate	1	S	S	W/E	E	
	3	S	(paper strip lost)			
	6	S	S	S	M	M
	9	S	S	S	M	M

S - strong; M - moderate; W - weak; VW - very weak; E - extinct; I_1 - irregular pattern, 90% of material gone; I_2 - irregular pattern, 75% of material gone; / - gives individual responses when disagreement of the observers occurred.

disagreement occurred rather frequently between the two men with regard to the odor evaluations. This disagreement is noted in the table by slash lines which separate the individual responses.

Whatever time for safe reentry is chosen between the 8- and 48-hour points in Figure 5, the time is matched or bracketed by the anthracene quantities used. The phenanthrene quantities employed are useful only at the 8-hour point. The skatole on the plate matches the 22.5-hour point fairly well. The concentrations of skatole on paper cover the time span with about the same options as the anthracene. The 2-phenylethanol concentrations used were too strong and did not match well. The lowest concentration of β-phenylethylphenylacetate matches the 8-hour point fairly well, and the next concentration would probably have been in the useful range but this remains a speculation since the test paper was blown away by the strong wind.

Disappearance curves for the outdoor tests with carbofuran (Furadan) and azinphos-methyl (Guthion) are given in Figures 6 and 7. At the end of the 55-day test, only 3 to 4 percent of the initial deposit of carbofuran remained on the plant leaves but between 25 and 30 percent of the azinphos-methyl remained.

During this test, the visual agents (anthracene and phenanthrene) were exposed on glass plates, in Ethocel films on glass plates, and on glass fibers or paper impregnated with Ethocel and Vinylite VYHH solutions of the agents. The odor agents (skatole, β-phenylethylphenylacetate, and 2-phenylethanol) were exposed similarly. The exposures on glass plates and of Ethocel films on glass plates were unsatisfactory means due to rapid disappearance of the agents from the glass plates and the lack of durability of the Ethocel films on glass plates. The items consisting of the impregnated glass fibers or paper fared better but were not without serious problems. Skatole was found to be unable to endure prolonged outdoor exposure without suffering severe changes in the basic odor or premature loss of odor. Changes in the fluorescent color and character of the anthracene and phenanthrene strips rendered them unsatisfactory within the first 1 to 2 weeks of the test. β-phenylethylphenylacetate and 2-phenylethanol retained their characteristic odors during the entire 40 days they were exposed, but from the 3-week point on, the

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Fig. 6 The disappearance of Furandan from Euonymus plants

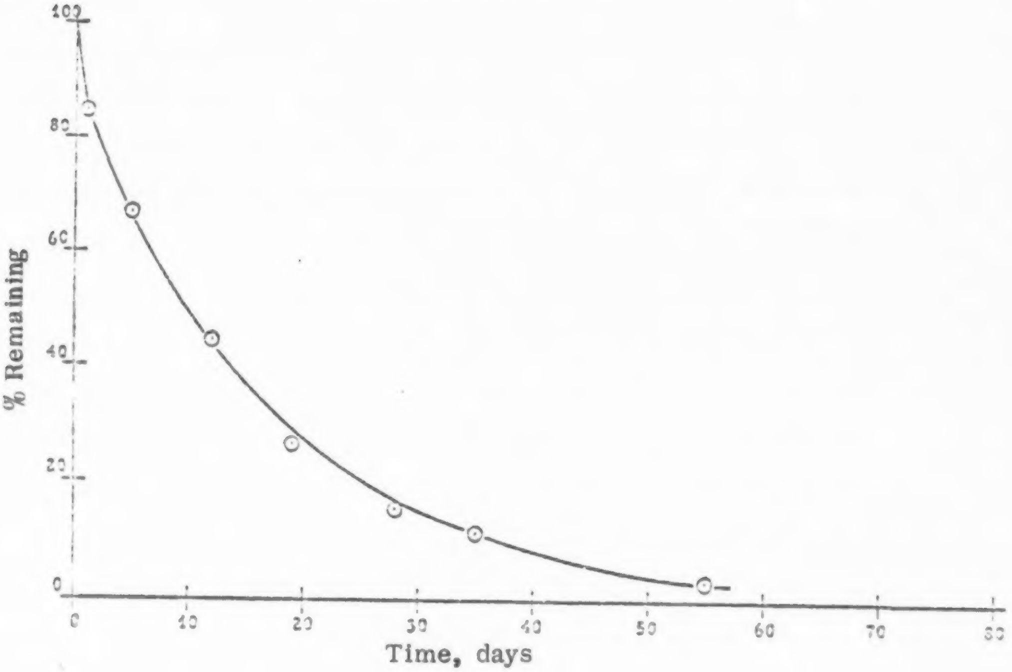
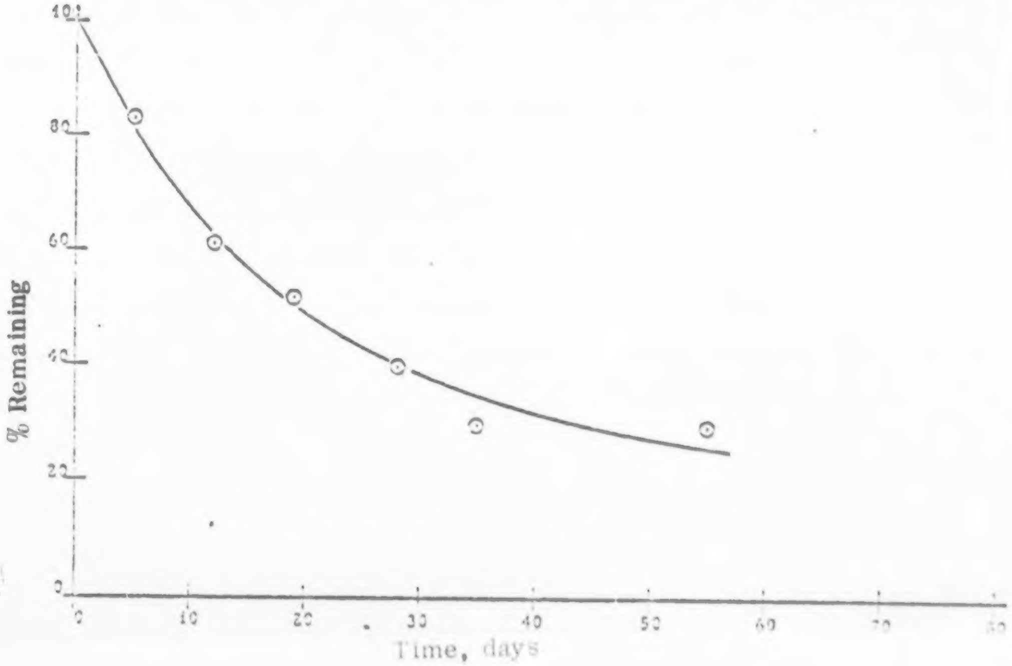


Figure 7. The disappearance of Guthion from Euonymus plants



odors weakened in such a gradual manner that distinct expiration points were difficult to perceive. At the end of 40 days, virtually all odor had left the items.

After the field tests were completed, a panel of five persons was exposed to three warning devices and interviewed regarding their opinion of the potential usefulness of the devices. Some of the panel did not detect the fluorescent light source immediately and many indicated that the light source was dim and not easily noticed. All recognized the color of the light source. Most of the panel said that a warning was indicated. Concerning the unpleasant smelling device (skatole), all of the panel detected a smell and most indicated that the smell was strong. All said that the smell was easily noticed and most said that a warning was indicated. Relative to the pleasant smelling device (2-phenylethanol), all of the panel detected a smell and most indicated the smell was weak. Most said the smell was not easily noticed and approximately one-half said that a warning was indicated. Virtually none of the panel preferred the pleasant smelling device (2-phenylethanol). The panel was evenly distributed between preferring the unpleasant smelling device (skatole) and preferring the visual device. All indicated that the concept would be useful for children, especially use of the unpleasant smelling device (skatole).

Cost Analysis

The cost of methyl parathion applications ranges from $2.50 to $6.00 per acre with the norm being $3.00 to $5.00 per acre. For conceptual Method 1, where skatole would be mixed with methyl parathion, the increased cost per acre is $90 or 18-30 times the present cost. For Method 2, where skatole would be used in two perimeter warning signs per field, the incremental additional cost is $11.00 per application. This cost as a percentage of the extremes of costs of application varies from 1 to 45 percent (see Figure 8). For Method 3, where anthracene or phenanthrene would be used in two perimeter signs per field, the total additional cost per application is $12.00. However, an additional cost is incurred in this method--the cost of viewing the treated sign with an ultraviolet light. It is assumed that a minimum of three readings will have to be made per application, resulting in a combined incremental additional cost of $15 per application. This total cost as a percentage of the total application cost is presented in Figure 9 and varies from 1.5 to 60 percent.

Figure 8. Incremental cost for odor device

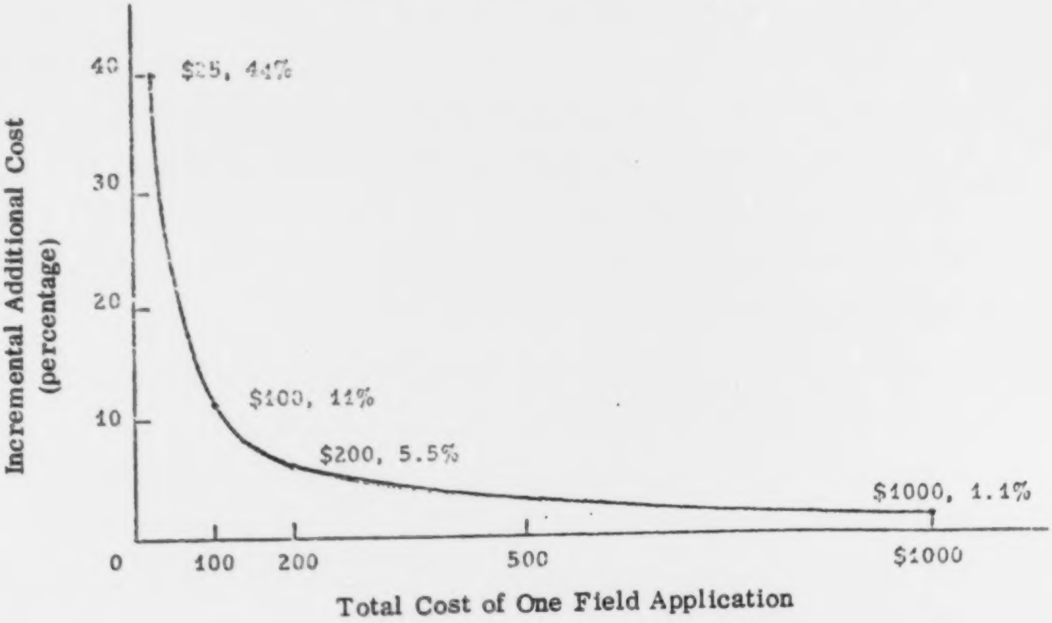
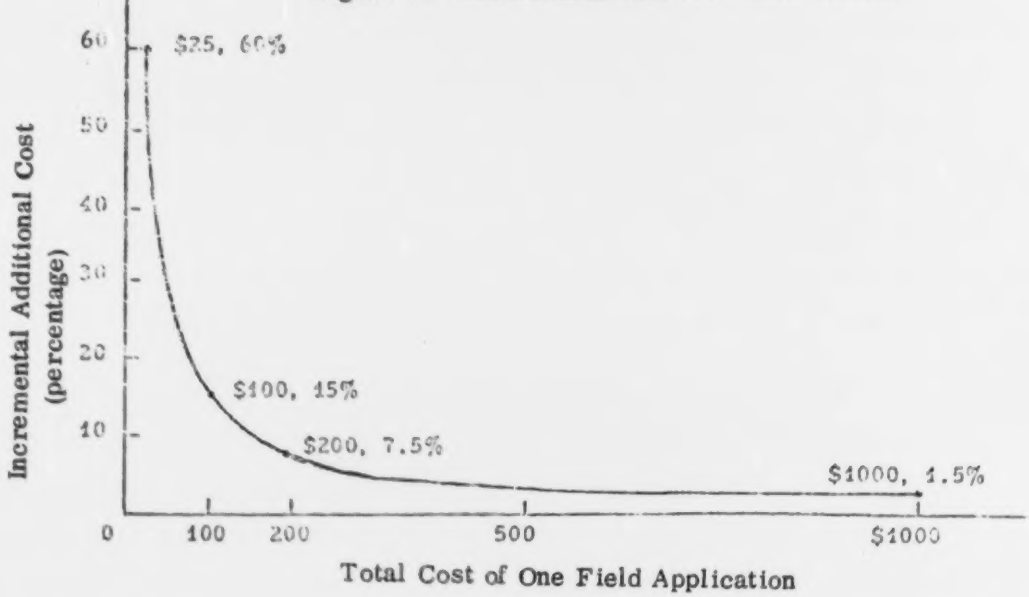


Figure 9. Incremental cost for visual device



Discussion

The odor agents finally chosen for work in this program were skatole, β-phenylethylphenylacetate, and 2-phenylethanol. The disappearance time for methyl parathion was matched quite well with the proper concentrations of the first two of these substances and not so well with the latter one. These substances, and probably most other useful odorant compounds, tend to tail off so that the end point as determined by human sniffing is not sharply defined. The disappearance times of Guthion and carbofuran were not well matched, generally.

Although the quantitative aspects of sensory odor agents are not as precise as desired, they do have a distinct advantage in that they are easily recognized by the general population. Even young children can be instructed that if they smell a certain odor that this means danger; thus, they should avoid the area. Some thought was given to the incorporation of odor agents in the insecticide formulation as an aid in preventing reuse of the formulation containers. This idea probably has to be rejected because of cost considerations and the difficulties which might be encountered in getting Government approval of adding a new chemical to the formulation.

In this program, attempts were made to develop sensory agents which would assist in preventing premature reentry into sprayed fields by not only farmers and field workers but also less knowledgeable persons such as young children. This program has clearly demonstrated that for insecticides with vapor pressures similar to methyl parathion, odor systems are feasible and provide a reasonable definition of the times when it is safe to reenter sprayed fields. For insecticides like carbofuran and azinphos-methyl, the development of useful systems requires more work but it does appear feasible.

The visual agents of importance to the program were polycyclic, aromatic hydrocarbons which fluoresce under ultraviolet irradiation. The compounds offering the best potential to this program were anthracene and phenanthrene. The disappearance of these substances, when used in properly selected quantities, matched the disappearance of methyl parathion quite well but did not match the disappearance times of azinphos-methyl and carbofuran. The end points of the visual agents tend to be sharper and more easily read than the end points of the odor agents.

The visual agents provide a more accurate end point because people have less variation in their sense of vision than they do for odors. These agents must be exposed to UV light in order to reveal their fluorescence. This requires more knowledge on the part of the user, and thus, it would be less useful for the general population. This program has demonstrated that for insecticides with vapor pressures similar to methyl parathion relatively accurate visual systems can be perfected. Additional feasibility studies are needed to evaluate visual agents for insecticides like carbofuran and azinphos-methyl.

The fact that the opinion survey was indecisive as to whether the visual sensory item or the unpleasant sensory item is to be preferred is compatible with the experimental results obtained when these items were exposed beside plants treated with methyl parathion. Only through additional experience could a distinct preference for either device be identified by objective or subjective means.

Warning systems, both visual and odorous, can probably be developed successfully for methyl parathion. With respect to the conceptualized methods for warning systems given in the preceding section, Method 1 is unacceptably expensive. Method 2 is less costly than Method 3. However, the deciding factor on which of these methods is best for general use is probably not a cost factor but, rather, that which evokes human response in the most effective and reliable manner. Both methods, from a cost standpoint, are acceptable for use in the cotton-growing regions where cost of application of methyl parathion is in the lower part of the range given. Probably neither would be acceptable in the higher part of the cost range given.

Comparison of the insecticide disappearance data from this program with that of other investigators has been made despite the difficulties resulting from dissimilarities in weather conditions, quantity applied, length of tests, plant species involved, and analytical procedure. In this program, the amount of methyl parathion remaining 3 days after application was 4.8 percent of the amount applied on the Euonymus plants and less than 1 percent on the cotton plants. Extremes found in the literature for the same time period were 23 percent (11) and 1.1 percent (12). In a similar comparison, but for a 4-day period, 86 percent of the azinphos-methyl was found on the plants in this program, whereas the high and low figures from the literature were 46 percent (13) and 16.7 percent (12). For carbofuran, the comparison had to be made after 14 days had passed. In this program, 39 percent was found to remain. Only one value could be found in the literature, and it was less than 0.4 percent (14).

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DR. LEWIS: Don, did it happen to rain during any of these tests that you were conducting?

DR. JOHNSON: No.

DR. LEWIS: I guess you didn't look into what might happen if it rained?

DR. JOHNSON: As a matter of fact, it did rain on one of our cotton plant studies, but it washed off all the methyl parathion, so the farmer resprayed this field a week or so later.

DR. LEWIS: This is what I was about to get to. I would think something like methyl parathion might wash off much more easily than either your skatole or anthracene.

DR. JOHNSON: For completing the study of this type, say this is a feasibility study, if you were going to carry this to a product, you'd have to do a good deal more climatic studies, before you'd say you had what you wanted.

DR. LEWIS: Here's just a comment for something I thought of, for what it's worth. One of your odoriferous agents coumarin could also be used as a fluorescence agent. If you dropped a drop of aqueous KOH on it and then exposed it to UV light, you'd get a pretty bright fluorescence there, you see. You have a double bi-functional method.

DR. JOHNSON: Yes. Coumarin doesn't give a very good odor, though, unfortunately. It's not sharp enough for us.

DR. ZWEIG: Are there any other questions? Comments? Someone on the feasibility of this idea? Anything new from Dr. Durham on reentry times?

See, the problem with the test is that we really do not know what the level of parathion is at which it is safe to enter, and I think Dr. Johnson made a pretty good guess at saying that if 90 percent of the methyl parathion is gone, he presumes that it would be safe to reenter.

However, once toxicological studies will have been conducted and completed, I think that -- if I'm correct, Don -- your visual or olfactory agent strip can be tailor-made for different reentry times.

DR. JOHNSON: Yes, particularly for pesticides. This is particularly true for the pesticides like methyl parathion.

Obviously, we've carried our feasibility study much further for that one, so we know that you can vary the disappearance times fairly accurately in those short time intervals, up to a couple of days.

So, even other compounds -- say it's 12 hours or 36 hours -- could be manufactured in fairly close tolerances.

ANALYSIS OF PESTICIDES AND PESTICIDE METABOLITES BY ESR HYPERFINE LABELING

Dr. Barry Commoner *

It is clear that the last paper was a test of an idea, and ours too is a test of an idea. The idea was simply this, that clearly in the Pesticide Program there was a serious problem of detecting and characterizing, and if possible, identifying the numerous complex and often unknown aromatic compounds — many of them quite similar to others — isomers and so on. The methodology for that has been based largely on such things as gas chromatography with mass spectrometry attached to it. It is cumbersome. It is difficult to separate isomers. Sometimes various kinds of errors come into it.

Several years ago, we at Washington University — Dr. Vithayathil; Dr. Weissman, a physical chemist; and I — got the idea that it might be possible to develop a whole new type of analysis, particularly for aromatic compounds by converting the compound of interest into a free radical. That can be done by either adding one electron or taking one away, by controlled reduction or oxidation. The moment you have a molecule with one unpaired electron, the paramagnetic properties of the unpaired electron can be detected by the technique called electron spin resonance. It is simply a form of spectrometry in which you place the material into a magnetic field; the unpaired electrons, being like little magnets will be aligned in two orientations toward the field, one with a north pole pointing south and the other with a north pole pointing north. The energy levels of these two populations differ, and the energy gap is a function of the magnetic field. You can set up a resonance absorption of energy, providing you have the right frequency, which for a typical Magnet of 3,000 gauss turns out to be in the radar field. Actually, it is hard to vary the frequency in this field, so we do the reverse. You fix the frequency of the radiation, then simply sweep through the magnetic field. So if you have an unpaired electron present — using X-band radiation which is a typical

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*Center for the Biology of Natural Systems, Washington University, St. Louis, Missouri pesticides of various kinds to animals and then looked for residues of the pesticides in urine, blood, and feces. We are nearly finished with this, and we are in the reporting phase. I think you can get a pretty good estimation of what the technique will do.

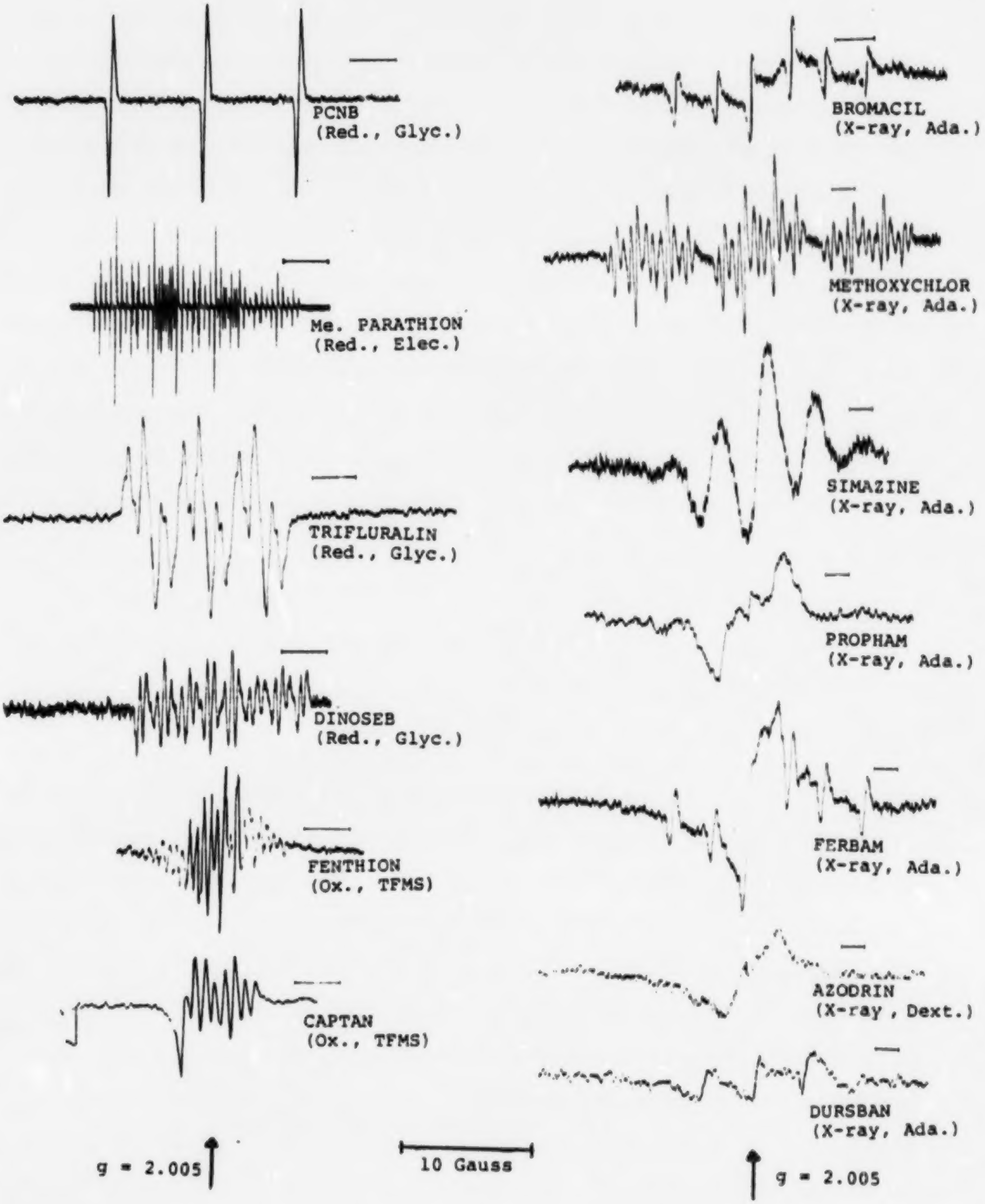
Figure 1 shows the ESR spectra of various pesticide free radicals we have tested. Now, let me pick out a typical free radical, for example, that of Azodrin. The way the scan is achieved is that the magnetic field is varying slowly in the horizontal direction. The signal which we see here is the first derivative of the absorption of the microwaves. In the case of Azodrin it is mainly just one signal here; that is an unpaired electron, without much splitting. We call the splitting "hyperfine splitting." That goes back to the history of spectroscopy, and hyperfine splitting is indicative of the molecular structure.

Now let us look at Figure 1 starting from the top. The first compound is PCNB. In other words, it is a free radical of a pure preparation of PCNB, obtained by reduction with glyceraldehyde under alkaline conditions. What we have here are three sharp peaks which appear at set magnetic field intervals. The next compound is methyl parathion (reduced now by an electrochemical technique that I am going to describe later on). This is a very elaborate series of hyperfine splittings. Reduction of trifluralin by glyceraldehyde under alkaline conditions yields a signal which has a triple pattern which repeats itself. Dinoseb also yields a signal with several splittings upon reduction with glyceraldehyde. Fenthion and captan yield distinctive signals upon oxidation with trifluoromethane sulfonic acid (TFMS).

Now, there is a group of compounds whose free radicals are formed by a technique that we tried out, which involved irradiation with X-rays. If you put X-rays through a material you can knock electrons out, or add them in, and you are never sure whether you are going to oxidize or reduce. Normally this does not give hyperfine splitting because if you work in solution, the free radicals interact very quickly. If you work in a solid, the electron is smeared through

Figure 1

ESR HYPERFINE SPECTRA OF FREE RADICAL FORMS OF DIFFERENT PESTICIDES



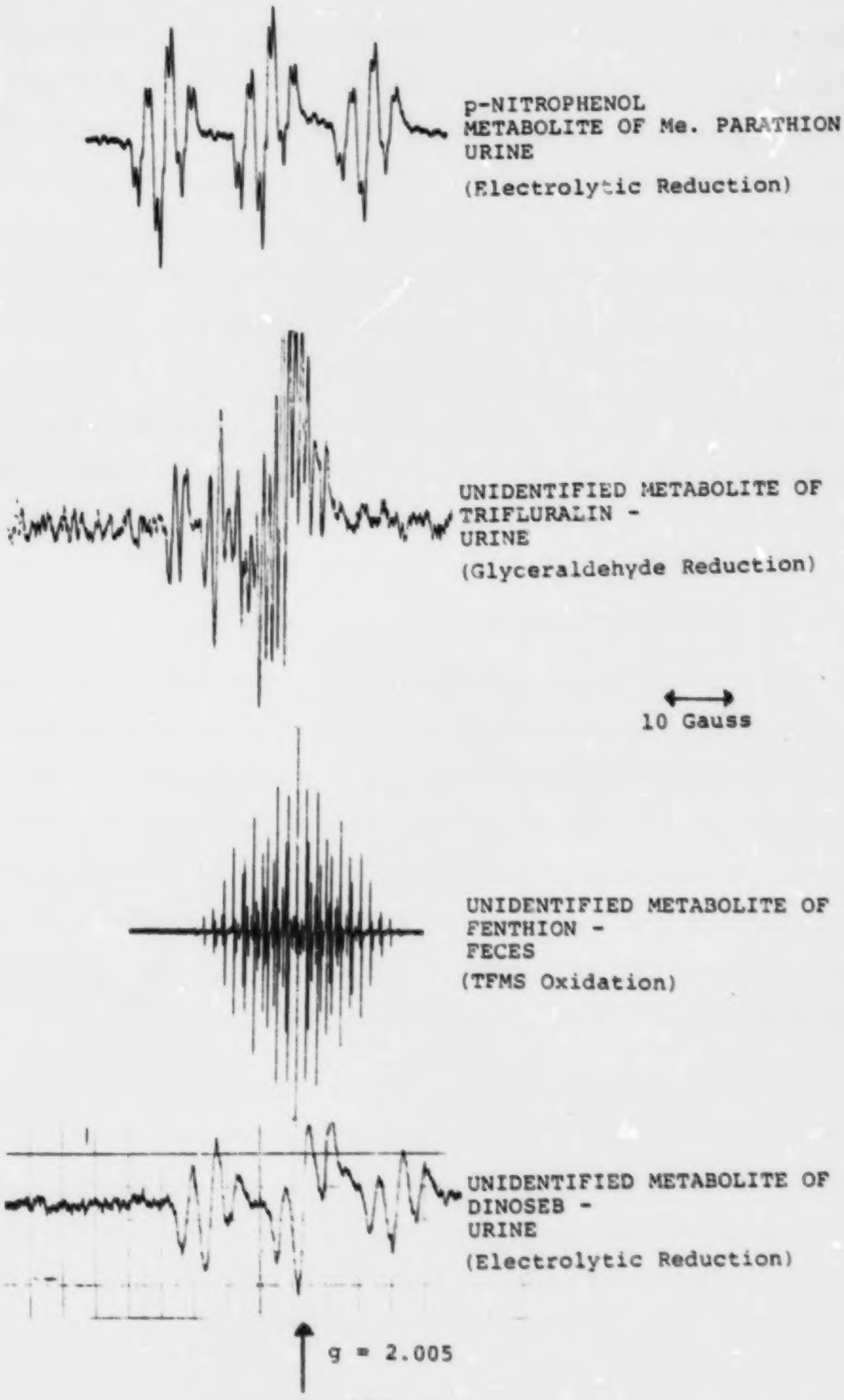
the whole structure, and you don't see a very definite thing. However, there is a unique technique in which it is possible to put a compound -- molecule by molecule — into a cage and them X-ray it. This can be accomplished by using a compound like adamantane, which is a three-dimensional hydrocarbon. In practice you make a joint crystallization, for example of bromacil and adamantane, and the bromacil molecules end up caged in the adamantane. We then X-ray it, making free radicals of the bromacil molecules, but since they are caged, they can't interact. This is bromacil simply X-rayed in adamantane. In other words, all you do is reprecipitate it with the adamantane, dry it, X-ray it, and then look at the ESR signal. In the same manner we have obtained signals from methoxychlor, simazine, propham, ferbam and dursban. Using a slight modification of this technique, i.e., X-irradiation in polydextrin, we were able to produce the free radical of Azodrin. Polydextrin, which is a short chain starch, is capable of making helical cages around compounds which will crystallize in the helix.

These are simply to show that one can make up an album of such signals which could be used to identify the compounds. All these are down at levels of about 0.5 to 1 microgram of material in the instrument, and I must say, the instrument can be made much more sensitive than this. These are quick runs. If you did slower runs of about 15 or 20 minutes the sensitivity could be enhanced.

In Figure 2 we see the free radicals of several metabolities of the pesticides that we have been working with. The first signal is that of p-nitrophenol, a metabolite of methyl parathion, which turns up in the urine of rats fed methyl parathion. This signal has a characteristic pattern. The second signal is from the urine of rats administered trifluralin. This signal also has an interfering signal superimposed on the signal due to the as yet unidentified metabolite of trifluralin. The third signal in Figure 2 is that of an unidentified metabolite of fenthion, which we find in the feces of rats that have been fed fenthion. We have not yet tried to identify it. The emphasis in our contract was not on making final identifications, but in characterizing them. The last signal here is that of an unidentified

Figure 2

ESR SPECTRA OF METABOLITES FROM RATS ADMINISTERED DIFFERENT PESTICIDES



aviation type of radar — if you use that kind of radiation at 3,000 gaus, you will get a resonance absorption.

The reason this is very useful as a means of identifying and characterizing complex compounds is that the magnetic moment of the electron interacts with the magnetic moments of certain nuclei. For example, the nitrogen nucleus is also magnetic and, as the electron swims through the molecule, it senses those paramagnetic nuclei with which it comes in contact and gives characteristic splittings of the signal. One can get very elaborate split signals, and the pattern of splitting, the spacing, the height, the number of them, etc., are so characteristic of certain types of structures that an experienced individual can even simply look at a structure and begin to say, "Well, yes, this is a nitrogen and there are two hydrogens near the nitrogen, and so on." Apart from that, of course, one can make known patterns and then recognize them very easily. That was the basic idea that we thought of — developing techniques for making free radicals out of substances of interest, and then showing whether or not we could detect them and characterize them.

One of the other advantages is that it's a very sensitive technique. In our lab, we've obtained as low as 10^{-7}, 10^{-8} molar free radicals and detected them. There is, on the horizon, a modification of the technique, a Fourier Transform electron spin resonance instrument, which will probably improve things by two orders of magnitude. It is inherently a very sensitive thing. It is pretty fast, and the most important thing perhaps is that we thought we might be able to use it to analyze mixtures without making any physical separation. I'll explain that point when we get to it. At any rate, this was the basic idea.

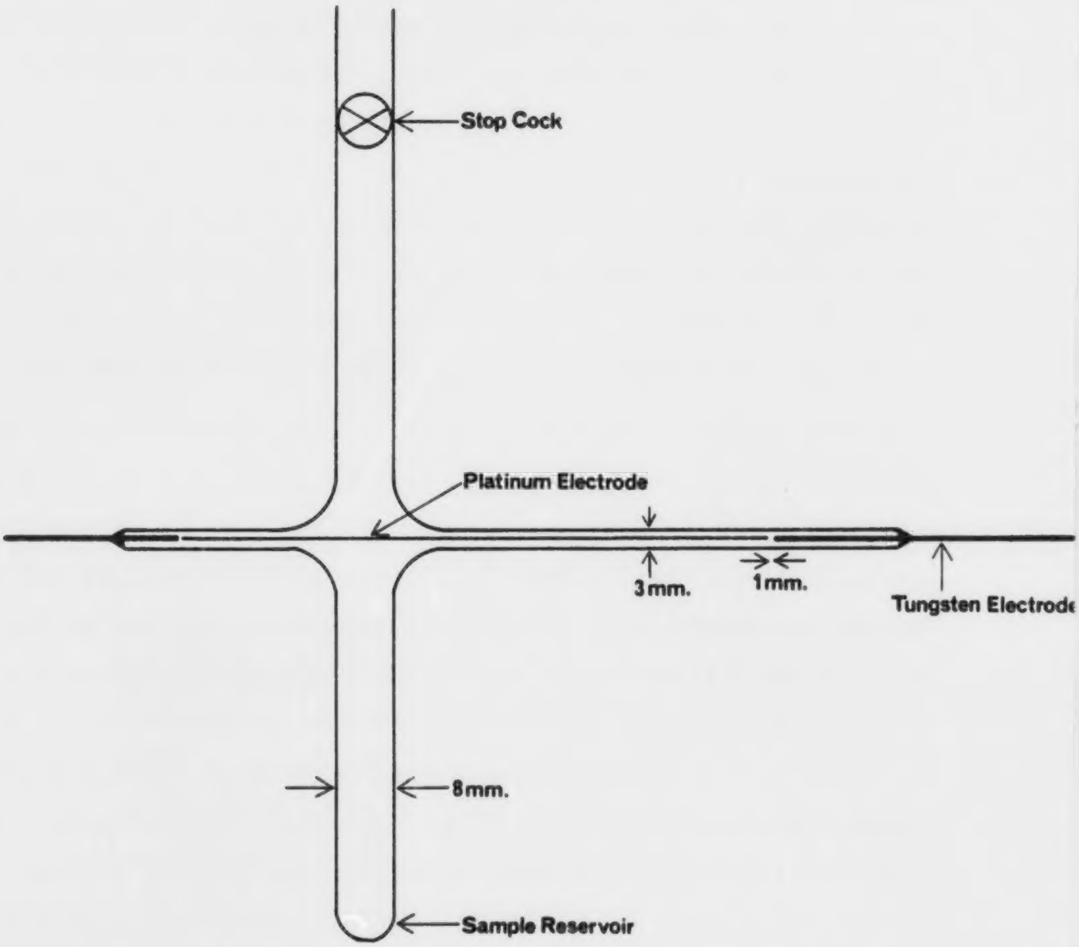
In discussions we had with the Pesticide Program it was decided that it would be a good test to see if we could find and characterize pesticide molecules in pure substances initially, but, in particular, in the bodies of animals that had been fed pesticides, particularly to see if we could pick up metabolites, as well as the pesticides themselves. Besides doing some basic studies, working with pure substances, we did a series of experiments in which we fed technical-grade metabolite of dinoseb which you find in the urine of rats fed dinoseb. It is not going to be terribly difficult to identify either of these compounds as soon as we get around to it. What this shows is that one can get characteristic, hyperfine signals from pesticides and from these metabolites that we have picked up in pesticide-fed rats.

Now I want to talk about the electrochemical technique of producing free radicals, because it turns out that this has certain remarkable advantages which probably will make it the method of choice. Incidentally, free radicals normally interact very readily with oxygen, so usually all of this work has to be done under high vacuum. For example, when we reduce with glyceraldehyde under alkaline conditions, the reactions are carried out in an evacuated cell. We make these cells ourselves; any glassblower can do it. In other words, the reactions are done under high vacuum, and the analyses are made under high vacuum except for the adamantane. The latter are usually quite stable and handle very well.

Now, in addition to the chemical and X-ray techniques described above, the oxidation-reduction reactions can be done electrochemically. Again, it has to be under high vacuum, and the cell we use is shown in Figure 3. There is a stopcock and that goes to the vacuum line. You can see there is a small gap between the platinum and tungsten electrodes which is the part that goes into the ESR spectrometer. The cell also has a reservoir for the sample. The method is very simple. We simply dissolve the material in a solvent such as methanol along with a carrier electrolyte such as tertiary butyl ammonium persulfate (TBAP), evacuate the cell, and apply an electromotive force (EMF). The EMF at which the free radical will be formed is a function of its redox potential. Since different substances have different redox potentials, we thought there was the possibility of selectively forming free radicals in the mixture by adjusting the EMF so that you could literally titrate and bring in one free radical at one EMF and another one in at a separate EMF and so on. That is the great advantage of this and, I must say, we were pleasantly surprised to find how well it worked.

Figure 3

ESR Electrolytic Cell


Stop Cock
Platinum Electrode
3mm.
1mm.
Tungsten Electrod
8mm.
Sample Reservoir

In Figure 4 we see the ESR spectra obtained from pure preparations of PCNB and 2-amino-4-nitro-6-secbutylphenol. The latter is a metabolite of dinoseb. Both of these compounds yielded characteristic spectra which could be easily distinguished from each other. Figure 5 shows the result of electrochemical reduction of a mixture of these two same compounds. In other words, equimolar mixtures of these two substances are taken up, put in the electrochemical cell, and analyzed. Initially at -0.8 volts all you see is the PCNB free radical; although the other compound is in the solution, you don't see it. We raise the voltage to -1.4, -2.0, -2.25 and -2.3 volts. As you increase the voltage you can see that the PCNB signal weakens and as we go from -2.25 to -2.3 volts, we suddenly see the 2-amino-4-nitro-6-secbutylphenol free radical. In other words, in this solution, simply by altering the EMF, we can bring one free radical into play and then the other one.

Originally we thought that as we increased the EMF we would get the second signal superimposed on the first, and we were prepared to do a computer subtraction. But, in many cases, as you continue the redox process, the first free radical goes over to a fully reduced or oxidized form and disappears. And, in this case it worked out that way quite nicely. So, this is an artificial mixture to show that the electrochemical separation really works.

Another example of electrochemical separation is shown in Figures 6 and 7. Here we are dealing with methyl parathion and its metabolite p-nitrophenol. Figure 6 shows the spectra of free radicals obtained from pure preparations of methyl parathion and p-nitrophenol. Figure 7 shows the results of stepwise electrochemical reduction of a mixture of these two compounds. At zero volts there are no signals. At -.25 volts the methyl parathion signal turns up. As you go to -.75 volts you still see it. At -1.35 volts it disappears. Now as you lower the voltage you see the p-nitrophenol signal. So here, without separating them, just by changing the voltage you see first one free radical and then the other.

Figure 4

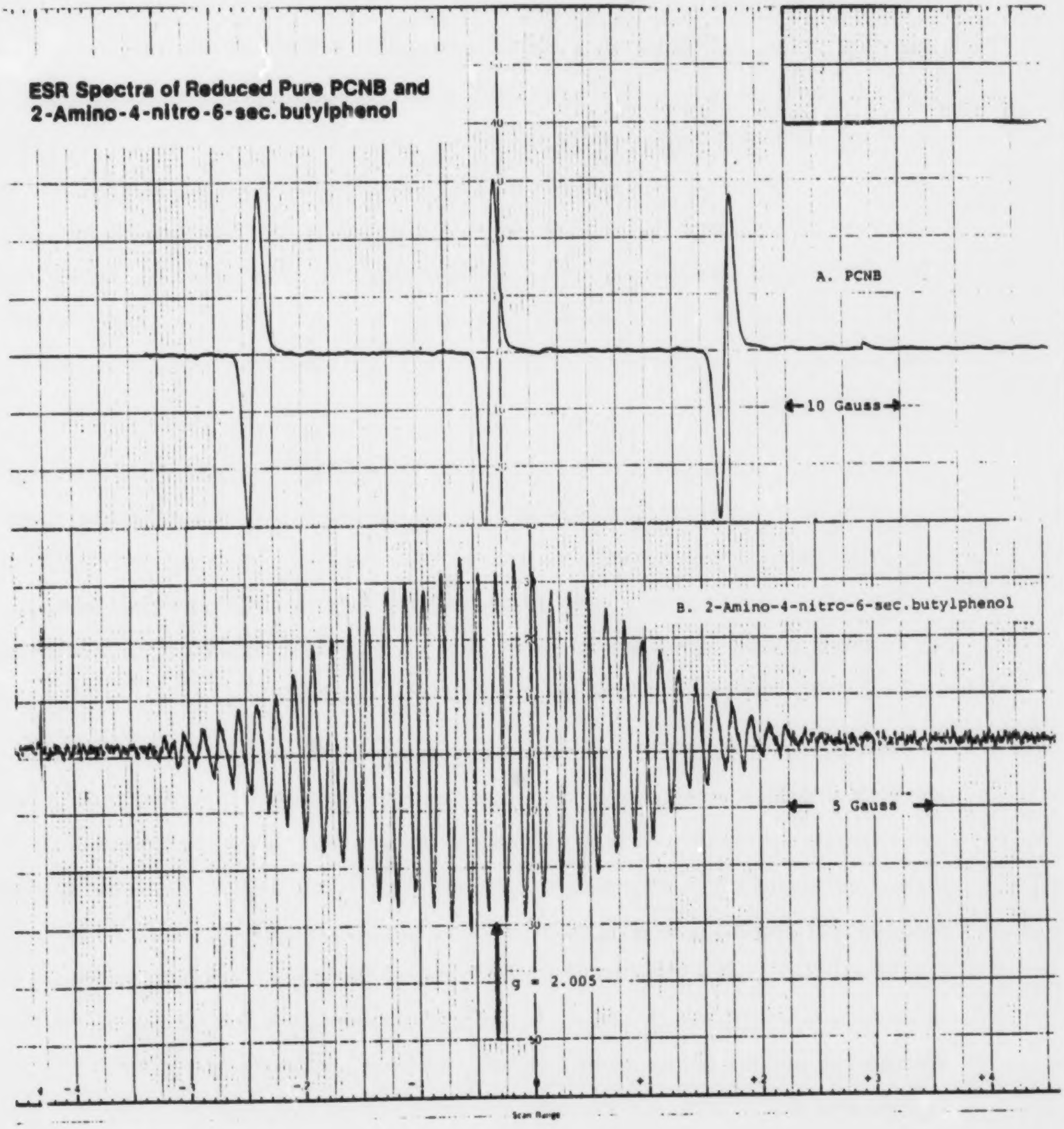

ESR Spectra of Reduced Pure PCNB and 2-Amino-4-nitro-6-sec. butylphenol

Figure 5

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Stepwise Electrochemical Reduction of a Mixture of PCNB and 2-Amino-4-nitro-6-sec. butylphenol

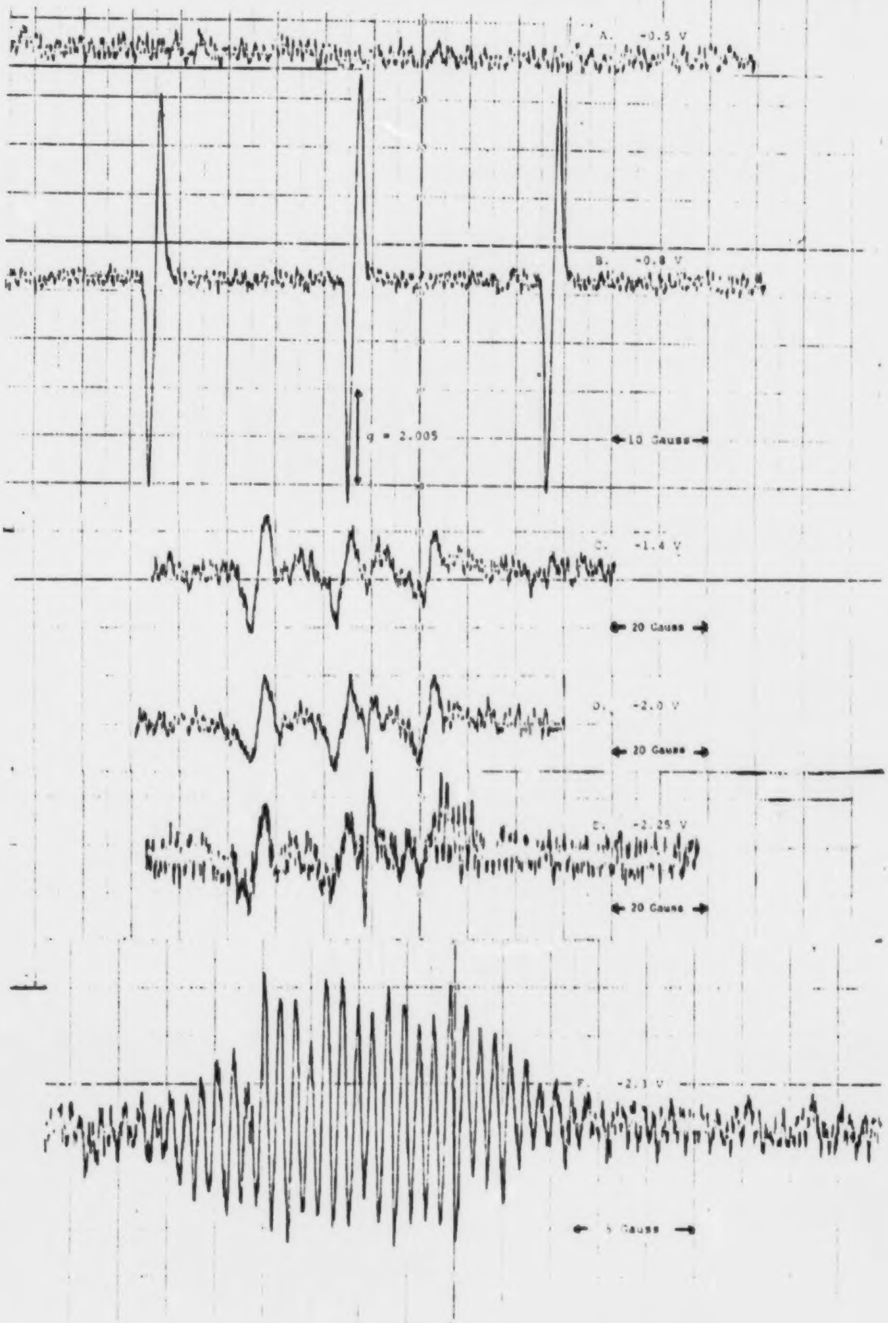
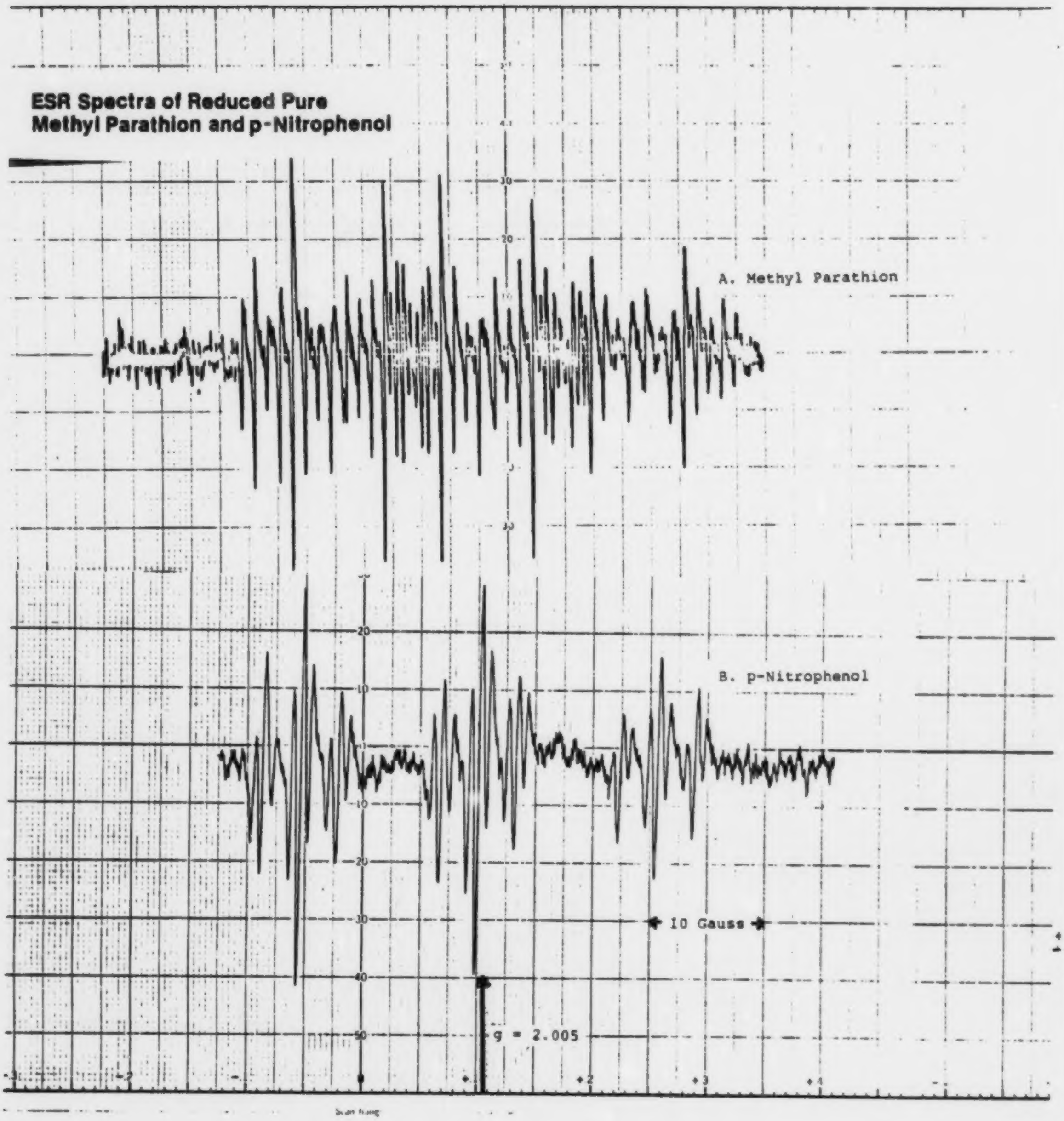


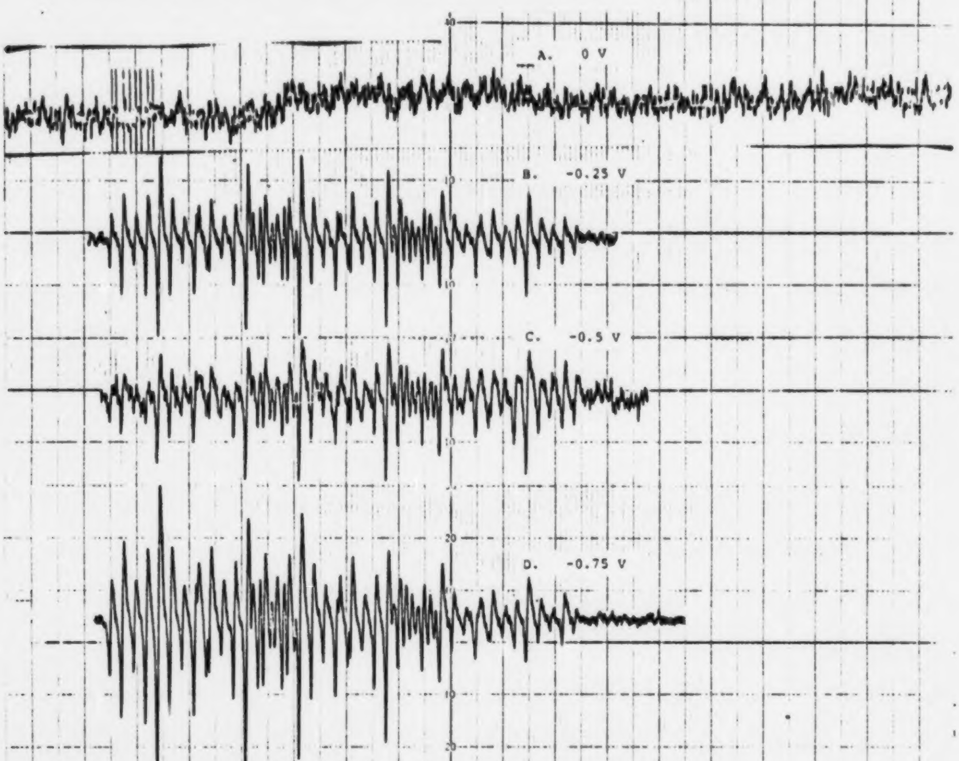
Figure 6



ESR Spectra of Reduced Pure Methyl Parathion and p-Nitrophenol

Figure 7

Stepwise Electrochemical Reduction of a Mixture of Methyl Parathion and p-Nitrophenol



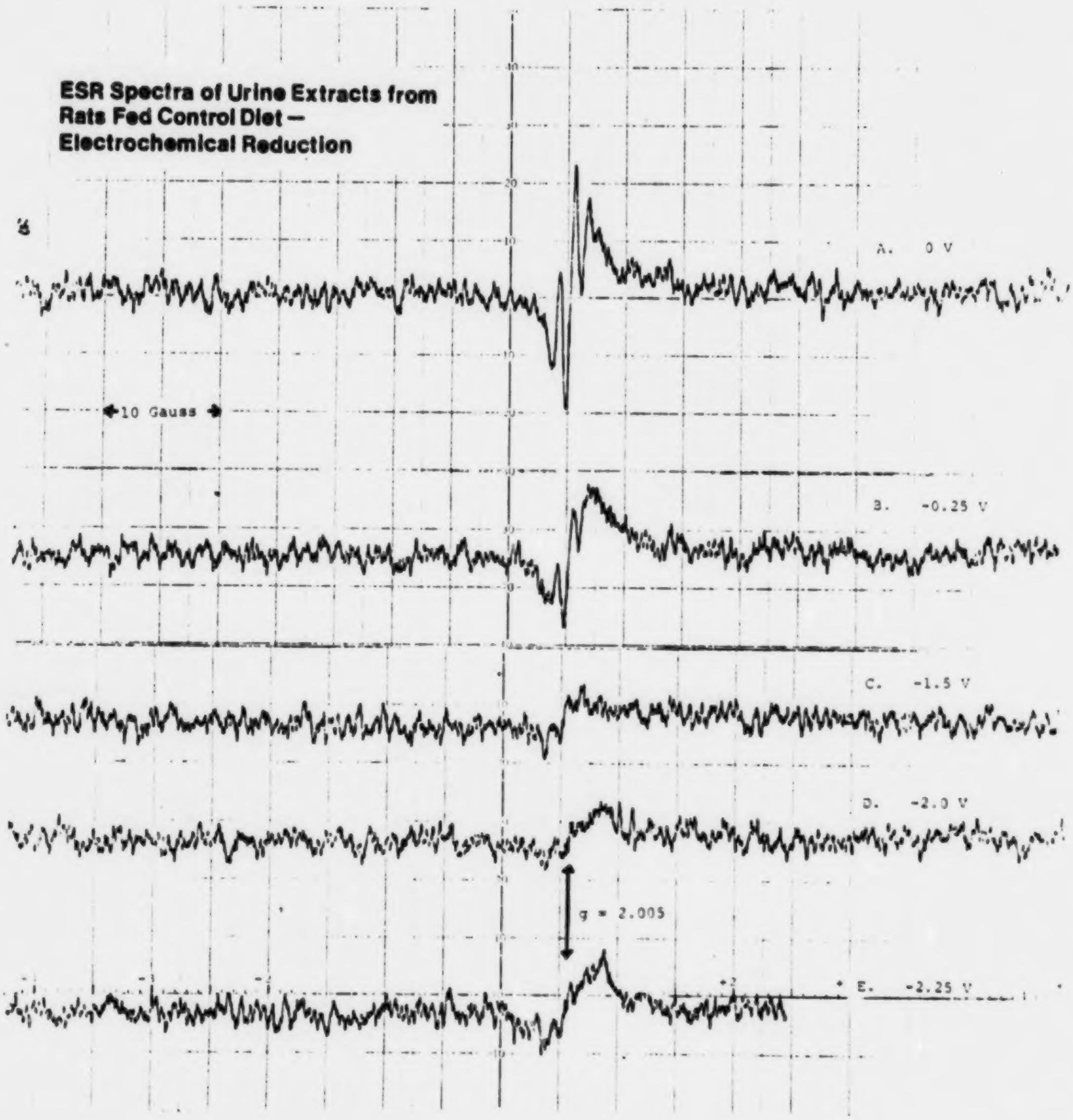
Next we will see how to use this technique in analyzing urine from rats that have been fed various pesticides. Figure 8 shows the results obtained from urine from a group of rats on a control diet. First, let me tell you what the sample preparation procedure is. The urine (25 ml) is extracted with hexane, the extract is taken to dryness, and the residue is simply taken up in methanol with the carrier electrolyte TBAP and placed in the cell. Figure 8 shows an interference signal. This turns up in urine and sometimes in feces. It is a well known free radical, ubiquinone, which arises from the diet of the rat. So there it is and that is at zero volts. Sometimes you get the free radical form simply from the carrier solution. As you make it more negative, this is -0.25, -1.5, -2.0 and -2.25, it gradually disappears. So this is our background signal, if you like, of urine from a control-fed rat.

Figure 9 shows the results from urine of dinoseb-fed rats. At zero volts here is the ubiquinone signal, and as you go down, the ubiquinone signal is still there but now a new signal is coming in at -2 volts and -2.5 volts. We have a pure signal of this as yet unidentified metabolite of dinoseb. In other words, the interference signal has disappeared yielding a rather clean signal of a metabolite of dinoseb. We have no doubt that we will be able to identify the structure of this metabolite.

Figure 10 shows a similar run for urine from a rat that has been fed methyl parathion. At first we see the ubiquinone signal which disappears as you apply the EMF, and in comes p-nitrophenol, which is very readily identified. And this is simply a rat that has been administered this pesticide in the diet and whose urine has been collected and analyzed in a rather simple way.

In addition to the examples given here, we were able to obtain PCNB signals in the urine of PCNB-fed rats and fenthion metabolite signals in the feces of fenthion-fed rats. To summarize, it looks to us as though this is a method which can be extremely useful in characterizing pesticides and their metabolic products in biological and environmental materials. There is no reason why it shouldn't

Figure 8



ESR Spectra of Urine Extracts from Rats Fed Control Diet — Electrochemical Reduction

Figure 9

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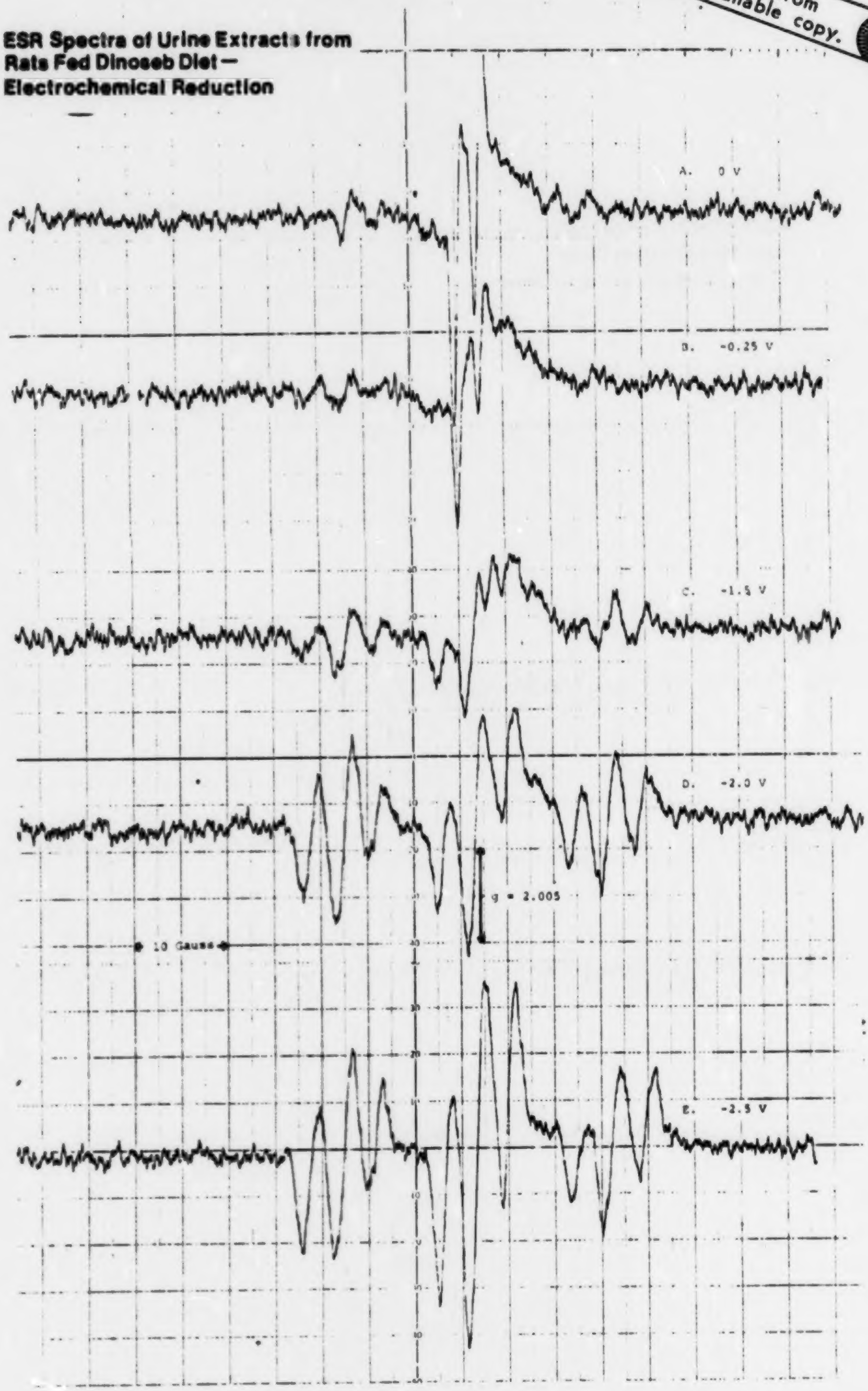
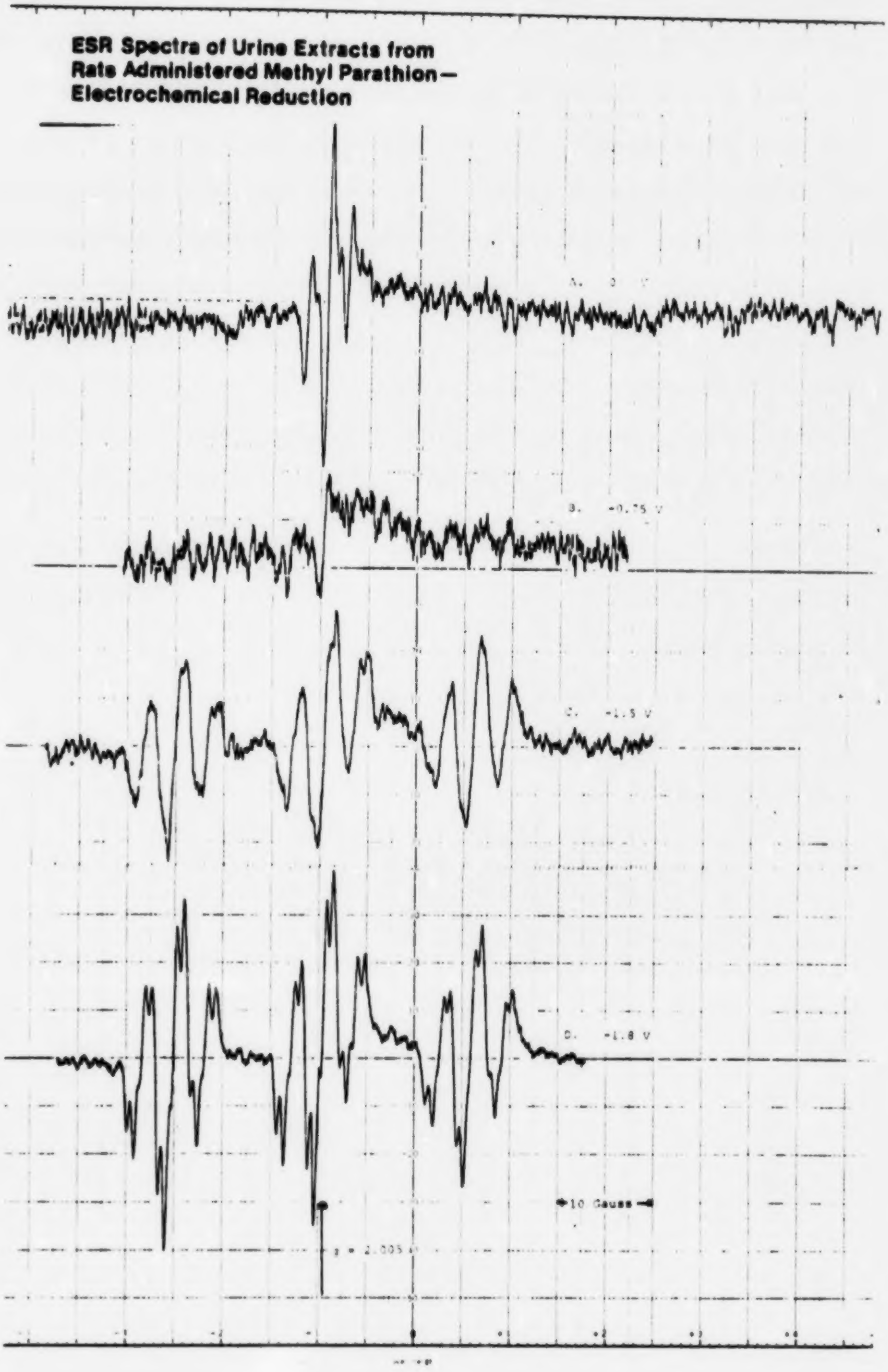


ESR Spectra of Urine Extracts from Rats Fed Dinoseb Diet — Electrochemical Reduction

Figure 10


ESR Spectra of Urine Extracts from Rate Administered Methyl Parathion—Electrochemical Reduction

work on soil, on tissue samples, etc. The remarkable thing about this is that it takes rather little cleanup to get to the point where you can do a good analysis, and, as I say, it is inherently a very sensitive technique. As some of you know, it is analagous to nuclear magnetic resonance, and in that case that technique was very much enhanced in its sensitivity by developing a Fourier Transformer instrument. Several of us at the University have been thinking about building the first Fourier Transformer electron spin resonance machine, and in fact, rudimentary parts of it are now kicking around between our place, chemistry and physics. It looks as though it is going to work. If it does, the entire sensitivity of the process at the physical end will be enhanced at least 50-fold, probably 100-fold, which means that the thing will become much more sensitive than it now is and it is already down in the range of parts per billion anyway.

Therefore, we do feel that we have in hand an idea which has been reduced to practice, and that it is something that can be very useful in the Alternative Pesticides Program.

MASS SPECTROMETRY METHODS DEVELOPMENT

Dr. Edward O. Oswald*

The first area which I will discuss is mass spectrometry methods development. Within the Environmental Toxicology Division of Research Triangle Park, we have two extramural efforts on the Substitute Chemical Program, which have been awarded during the later portion of FY-75. One of these efforts is a feasibility study investigating field ionization, mass spectrometry and its applications to investigating pharmacokinetics, metabolism distribution, excretion of pesticides, and biological systems.

The second area concentrates on interfacing a mass spectrometry system with high-pressure liquid chromatographic systems. Within the framework of this contract, which resides in the Analytical Chemistry Branch, this first of all concentrates on the design -- interfacing hardware to connect a high-pressure liquid chromatograph with a chemical ionization mass spectrometer. This particular award was made to Finigan Corporation, Sunnyvale, California. The project officer on behalf of Finigan Corporation is Dr. Bill McFadden.

DR. ROSS: In respect to field absorption, I think if you just hit on what is unique about this particular technique, and what you anticipate using it for, and on what particular compounds. In other words, what can be done with field absorption that cannot be done with chemical ionization, as well as electron impact mass spectrometry? That's my first question. I have another one when you've answered that one.

DR. OSWALD: That I expected. Numerous modes of ionization are utilized in mass spectrometry. As you bombard the compound with a stream of electrons, you have excess energy once you knock one electron. In numerous instances in the analytical mode, you are unable to detect the molecular species as you analyze in the molecular regions utilizing a high energy, such as 70 EV. In this instance you do have excessive energy which can bring about fragmentation of the molecules, minimize molecular species.

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*Health Effects Research Laboratory, Research Triangle Park, North Carolina

In the case of field ionization, you are depositing a sample on an edge or point. A high-energy field is produced at the same time the sample is vaporized and ionized. In the case of chemical ionization, an indirect mode of ionization is involved in that a carrier gas, whether it's methane, isobutane, or helium, first of all is ionized. The charged species of these is then transferred by high pressure, in this case in the ion source from the charged species to the solute, namely the compound of interest.

In the case of both field ionization and chemical ionization, the level of energy of transfer for the ionization mode is much less than that of the direct electron impact mode of ionization. So in this case you are looking at increased populations of the molecular species.

In respect to the field ionization contract, two compounds specifically considered for this program are parathion and trifluralin, both from the standpoint of metabolic investigations and inhalation studies. From the metabolic investigations the normal sequence of events as expected would be administration of the compound, analysis of biological fluids, excreter and tissues, using the analytical technique in this case, field ionization mass spectrometry.

I'll attempt later today to differentiate field ionization and field absorption. In the case of field ionization, there is a requirement of volatilization of the material. In field absorption there is not; it is also a low energy process. The requirement for vaporization in field ionization has advantages and disadvantages.

Going on further to elaborate on the field ionization contract, a difficult problem in inhalation measurements is that of having an in situ mode of analysis of compounds used in inhalation studies.

DR. ROSS: The FI technique has been used primarily for a long chain, hydrocarbons and things of this nature, more so than other environmental compounds.

Since field ionization, field absorption is available commercially, the uniqueness of the contract at Stanford University is a feasibility study for pesticides, the in situ studies as you were mentioning.

DR. OSWALD: That's correct. The other unique portion of this is development of the multipoint source system.

DR. ROSS: I am assuming that the purpose of this contract is to interface the liquid chromatograph. With these objectives you would like to be able to chromatograph those heat labile compounds. Assuming that you accomplish this, and once the compounds have been eluted along with the solvent to the source, do you know anything about the source, the stripping process they're going to use to get the solvent away from the compound, and also the limitations that it has once it reaches the source? Because one of the objectives of an interface system like this, if you're going to do a study with heat labile compounds, is to have minimum contact of the surface area once it reaches the ion source. And if I read the proposal correctly, it seems as if this is a derivative of the Scott method which used a heated wire. There will probably be maximum contact once it got into the source. Can you elaborate on this?

DR. OSWALD: The general technique of interfacing with LC systems, then removing the solvent from the eluting column prior to entrance into the ion source involves the following processes. A concentrating effect must be produced. And with most of these systems up to this point in time one percent or less of the LC effluent enters the ion source. One of the first criteria to be overcome and desirably to be included in this program is to increase the amount of solute which enters the ion source.

At this point in time a realistic figure is at least 30 percent. From the standpoint of concentrating the solute or totally eliminating the solvent, the mechanism used is very similar to that initial work described by Scott, in that a moving wire -- in the case of this particular contract, a modified ribbon or trough system -- is included. There are points of consideration which should be given in any system of this nature.

You balance the volatility of the solvent with that of the solute. As you go to very nonvolatile solvents, you'll always have a tendency of losing the solute. At the same time we were talking about removing all of the solvent in this mode of operation, not using the solvent as a mode of ionization, as was reported in other earlier investigations.

The flexibility of the system as proposed at this point in time is that from the Agency standpoint we felt that the system should be applicable to chemical ionization systems available within the Agency now, either the Finigan system or the Varian systems. The Finigan system, as proposed, would have flexibility to operate in addition to the CI mode in the electron impact mode; without removing hardware the same system could also operate in the GC/EI mode, and a GC/CI mode, in addition to the LC/EI/CI modes.

I'll try to clear up these abbreviations if there are any problems, without bogging down and using other time here.

DR. ROSS: With reference to the same question I just asked. Selection of the CI mode -- I think I understand, but several people here are not well-informed. The difference between electron impact and chemical ionization -- there are definite advantages of one method over the other. I think that maybe you should elaborate on the advantages of going to the CI mode in lieu of the electron impact, and since the CI mode and pesticide residue analyses really are still at a rather pioneering stage at this point, why we are pursuing this particular technique?

DR. OSWALD: To make the answer short, flexibility within the system. To elaborate on this, a system which is capable of operating in the mode of chemical ionization, namely an open, high-pressure source, can also be operated in an electron impact mode. Electron impact spectra do complement chemical ionization spectra.

Now, without getting into the technicalities too deeply, polyfunctional compounds, as I indicated earlier, have a great tendency to have low concentration of molecular ions formed in the direct electron impact ionization mode. In that you are taking a stream of electrons, bombarding a compound with this, knocking out an electron, and forming an ionized species.

In the case of a chlorinated species, depending upon the structure of the compound, you find a relatively high abundance of molecular species formed in an electron impact mode. In the case of certain biodegradables, where you've got polyfunctional nuclei, such as nitrogen, phosphorous, oxygen, and others, the tendency to form a molecular ion upon electron impact mode of ionization is very low. This means if you do not know the characteristics of the molecular species, you are limited on how much you can say, or even quantitate about the total unknown.

From the standpoint of considering why we approach the chemical ionization route versus electron impact of the modes, as I indicated earlier, it is because of flexibility. If we can solve the chemical ionization problem, the means of getting the solute into the ionization mode, it means minor adjustments in the reagent to simulate the electron impact-like mode.

For instance, a number of laboratories have taken just the chemical ionization mass spectrometer, using reagent gases such as methane or isobutane. In this case you are dropping the total energy level for the mode of ionization somewhere in the range of 15 to 20 electron volts. This means in order to break a carbon-carbon bond, which requires the equivalent of 12 to 20 electron volts, you do not have a large excess of energy available.

As you choose your gas, you also choose the amount of electron excess energy. If you want to look at it this way, you also increase the degree of ionization. Taking the other situation, you can substitute in chemical ionization mass spectrometry helium and get an electron impact-like spectrum, in that comparing a true electron impact mode where there is no reagent gas versus helium, you do see very useful characteristics which mimic electron impact systems or characteristics.

The flexibility and the complementation of chemical ionization and its capability from a chemical ionization standpoint gives a lot of advantages, more so than if we would have considered only the electron impact system mode of interfacing separately.

DR. ROSS: What about the complexity between the two types of spectra?

DR. OSWALD: If you read the literature -- and I'm one not to generalize but I've been involved in this particular area for a number of years -- and only after you do the experiment can you say in most cases the chemical ionization spectra are more simple in that there is less excessive energy fragmentation. From an interpretive standpoint, there are normally less ions present in chemical ionization spectra than there are electron impact spectra due to the level of excess energy available.

At the same time this is compound-dependent. Numerous compounds such as PCB have a threshold level for ionization. CI is not the most desirable mode of ionization for these types of compounds. You need more energy to virtually make a stable or to form a stable ion.

DR. ROSS: I have three very quick questions, and maybe you've already answered them. I didn't get them. One is, do you have to work in the mass spectrometry in the atmospheric mode? In other words, how do you interface a liquid column and a mass spectrometer? Two, how do you get rid of the solvent? And three, isn't it rather unusual that the Agency went with a commercial company? That, in a way, may be commercializing the products. Or is this a normal procedure?

DR. OSWALD: Let me take the question concerning the removal of the solvent. The removal of a solvent will be carried out by a stream of gas, namely nitrogen, and a temperature below that required to remove the solute but still sufficient to remove that of a solvent.

In regard to the question concerning interfacing the LC to that of a mass spectrometer where you're talking about pressure differences, in this case the interface does require vacuum lock systems, so that the engineering techniques are required. You're not working atmospheric pressure. You're working at comparable levels to that of normal chemical ionization mass spectrometer of 10^{-5} tor, or in that range.

In relationship to the contractor, at the time that this particular contract was advertised, the idea -- and the idea still remains -- was that the expertise needed to understand the hardware regardless of the system is the most important thing. You don't bring in a group which may have one expertise and try to fit it to a system with which they're unfamiliar. That's the hurdle on which we don't want to concentrate. In this particular case we singled out two mass spectrometer systems common to the Agency, which is not unusual. In this case there are approximately 35 or 40 systems.

QUESTION: Is that common?

QUESTION: Which are in the Finigan system?

DR. OSWALD: A number of laboratories do have the Varian systems. One of the criteria which, by the way, is included on this work scope is once this contract has fabricated and evaluated this system, two systems will be released to the Agency at the termination of this contract. One will be evaluated in our laboratory, and one will be evaluated in another one, for application to environmental problems.

QUESTION: I think you just answered my question, but do I understand that once the LC interface system is set up, you have not created a dedicated mass spectrum? That you can break that apart and use it for other purposes without a lot of cleanup and so forth?

DR. OSWALD: That's correct. In fact, the proposal includes that it will not interrupt other modes of operation, namely GC electron impact, administration of the samples through the probe. You still have the flexibility without taking the hardware off so that you can operate in the electron impact, the chemical ionization GC modes, and also the LC modes.

DR. HILL: This isn't a question, it's mostly a response to some of the previous questions as to a practical application of chemical ionization mass spec and pesticide work. Many of you may already be familiar with this, but Dave Stalling out in Columbia, Missouri is very interested in toxaphene in fish, and if you know the gas chromatogram of toxaphene, you know what that looks like, and you probably all heard of Cassda's efforts to resolve this into some 200 plus components.

Chemical ionization mass spec, coupled with GC, can take the seven fractions of toxaphene, the early cleanup Cassda's fractions, if you will. I've seen Dave Stalling's slides on this -- they're beautiful. It would be hopeless with conventional mass spec to try to interpret this because each of these fractions contains 20 to 25 components, but with chemical ionization you can clearly follow the loss of chlorine or the numbers of chlorine items from the more early eluting compounds up to the others, and you can get a fairly clear comparison of domestically produced toxaphene versus other brands from other countries, which is becoming an important issue in the toxicology of this material. So this is not a residue application but, after seeing those slides, I think this is the only way you could ever make any sense out of mass spectra of toxaphene.

DR. ROSS: Thank you, Dr. Hill. I've talked with Dave Stallings at the mass spec conference the other week about his work, with reference to that. I'm aware of the applications of chemical ionization. There are numerous people who have had questions related to chemical ionization and I thought this would be an opportunity to differentiate among the various techniques. But one thing which I could have been reading into what you said earlier, Ed, in the development of this contract -- you said an interface system which would work for the common systems found among EPA laboratories, which we named the Finigan and the Varian -- since this contract was let to Bill McFadden of Finigan Instrument Corporation, I would find it very strange that he would build an interface system that would also be adaptable to Varian instruments.

DR. OSWALD: The work scope reads either/and/or.

DR. ROSS: Also there are two different types of instruments, and I understand that the interface system has to be specific, either for your quadruple or for your double focusing.

DR. OSWALD: That's correct. Now this is the other complexity as you become involved with engineering of any system. From the standpoint of the work scope as proposed, it includes either the Finigan or the Varian systems. We did not have a company which would agree to tackle both with the available restricted level of funding.

AUTOMATED CLEANUP AND SPECIFIC DETECTOR SYSTEM FOR PESTICIDE RESIDUE ANALYSIS

Kenneth R. Hill *

This is a report on the status of an EPA-ARS Cooperative Agreement research project entitled "The Development of a Semi-automated Cleanup and Analysis System for Pesticide Multiresidue Analysis."

Although existing methods (AOAC, PAM, etc.) for the analysis of pesticide multiresidues, developed mainly for regulatory use on foods, have been very successful for a variety of pesticide/substrate combinations, there remain a large number of compounds (mainly OP and carbamate insecticides) which cannot be reliably measured by these techniques, especially in environmental samples. For example, of the P- and/or S-containing compounds in the priority list for the Substitute Chemical Program, only parathion, methyl parathion, and malathion have been determined by the AOAC multiresidue procedure and only the preceeding three plus azinphos-methyl (and ethyl), demeton and phorate have been determined by the Abbott procedure (1).

In each case the methods have only been proven for selected food groups and not for a wide variety of materials such as soils, waters, aquatic organisms, etc. An urgent need therefore exists to extend present multiresidue methodology to the pesticides on the priority list and to search for and develop alternate multiresidue methods which are more universal in scope and offer greater simplicity and/or speed, especially in the cleanup stage. This research project, based on recent discoveries and developments in the Analytical Chemistry Laboratory, ARS, is directed to the latter approach.

High-speed, high-pressure liquid chromatography is limited in application by the lack of selective detectors. One approach to overcoming this problem was made by combining the P- and S-selective flame photometric detector with the Pye traveling wire system, thus enabling liquid effluents to be vaporized, detected, and measured directly (2). This apparatus and its capabilities will be described briefly. Figure 1

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*Analytical Chemistry Laboratory, Agricultural Research Service, USDA, Beltsville, Maryland

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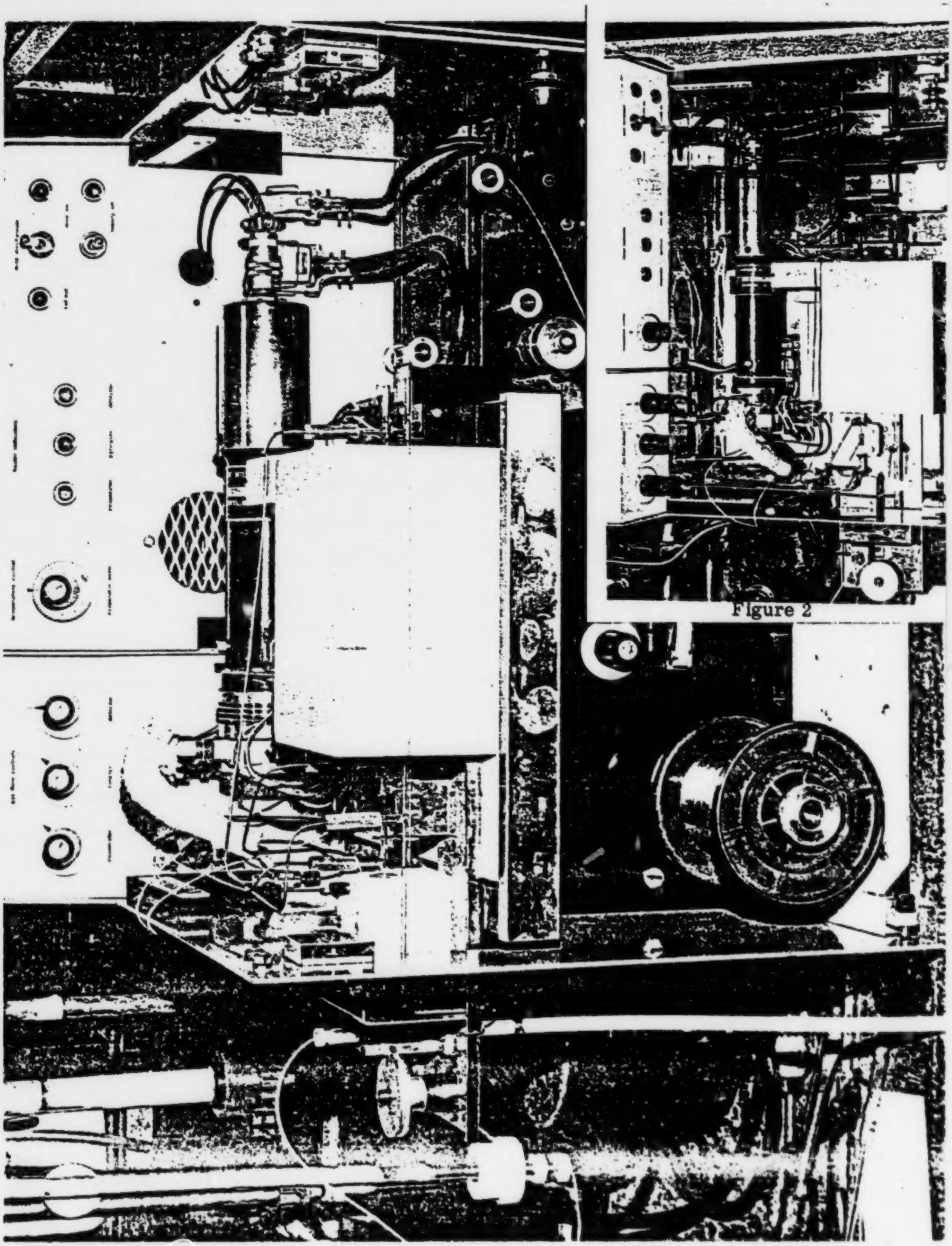

Figure 2

Figure 1

Figure 3

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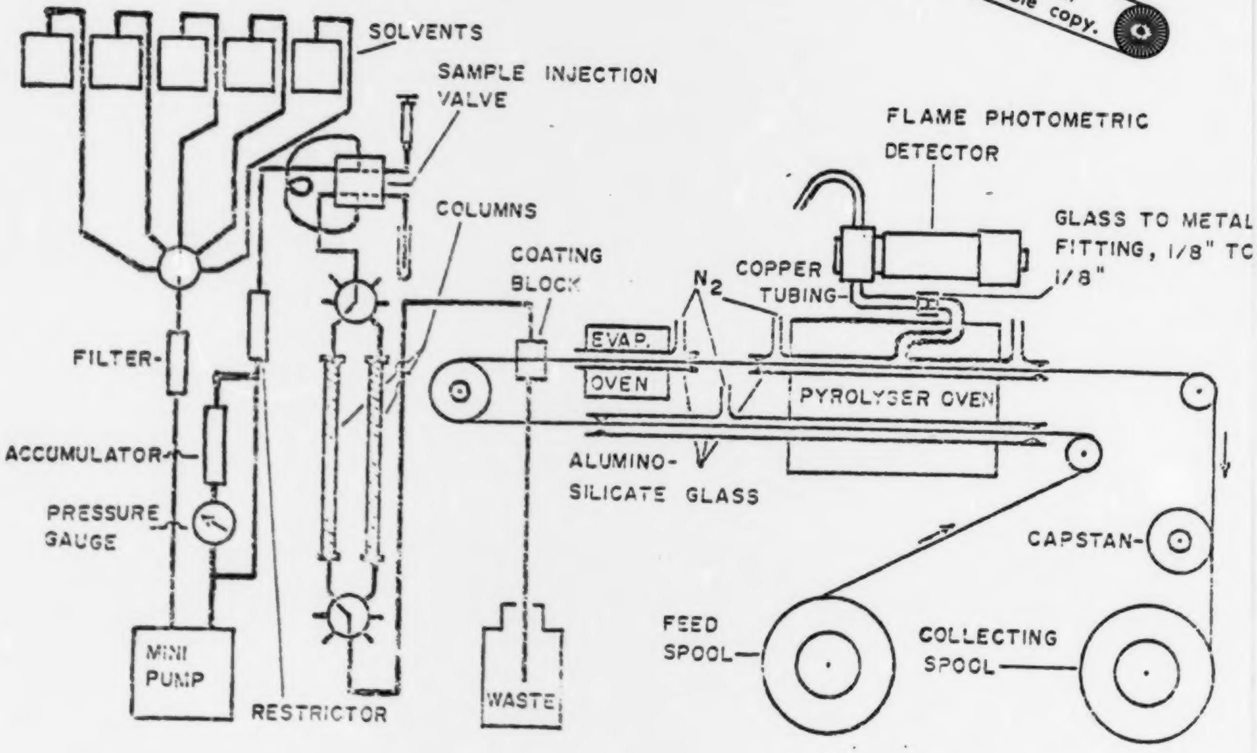

SOLVENTS
SAMPLE INJECTION VALVE
FLAME PHOTOMETRIC DETECTOR
COLUMNS
COATING BLOCK
N_2
COPPER TUBING
GLASS TO METAL FITTING, 1/8" TC 1/8"
FILTER
EVAP. OVEN
PYROLYSER OVEN
ACCUMULATOR
ALUMINO-SILICATE GLASS
PRESSURE GAUGE
CAPSTAN
FEED SPOOL
COLLECTING SPOOL
MINI PUMP
RESTRICTOR
WASTE

shows a front view of the Pye traveling wire system with a flame photometric detector installed in the back compartment. The installation is rather straightforward and requires only minor modifications to the Pye-Unicam system. Figure 2 shows a top view of the same apparatus. Figure 3 shows a block diagram of the apparatus and its associated liquid chromatographic columns. Figure 4 shows the linearity achieved with various concentrations of standard malathion over the range of 0.1 to 1 microgram per milliliter eluted from a Corasil II glass bead column at a flow rate of 4 milliliters per minute. Peak heights were linear with concentration with a correlation coefficient 'R' of 0.99966 and a slope of 23.9 millimeters per nanogram. Figure 5 shows the reproducibility that was obtained with repeated injections at the same concentration. Figure 6 gives the names and structures of several of the pesticides used to test

Figure 4. Standard Malathion

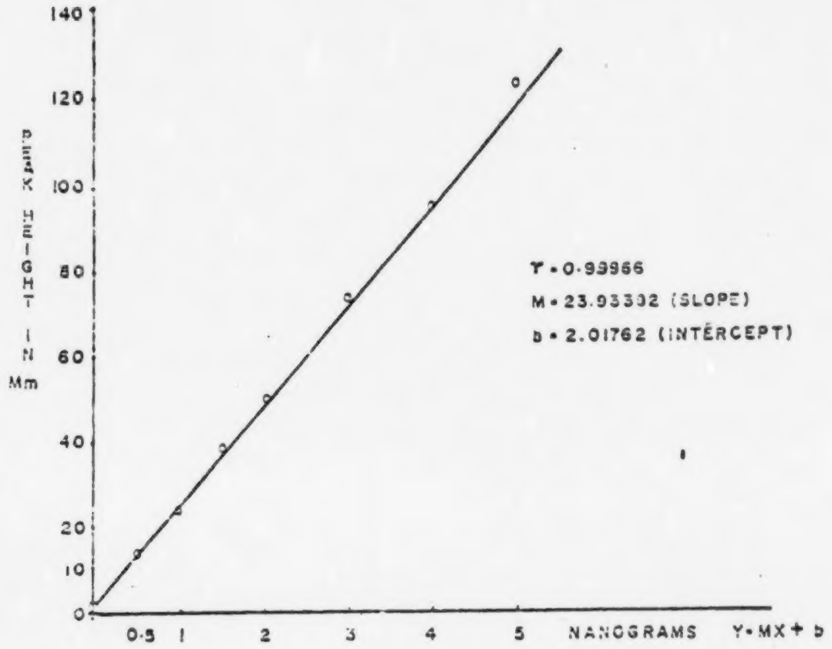


Figure 5

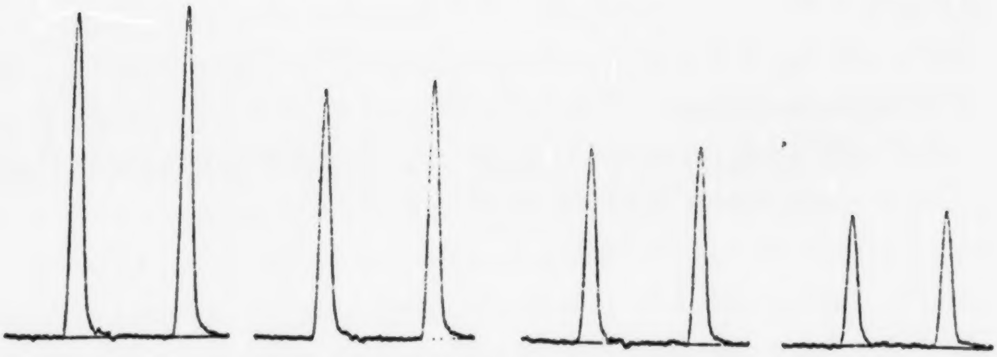
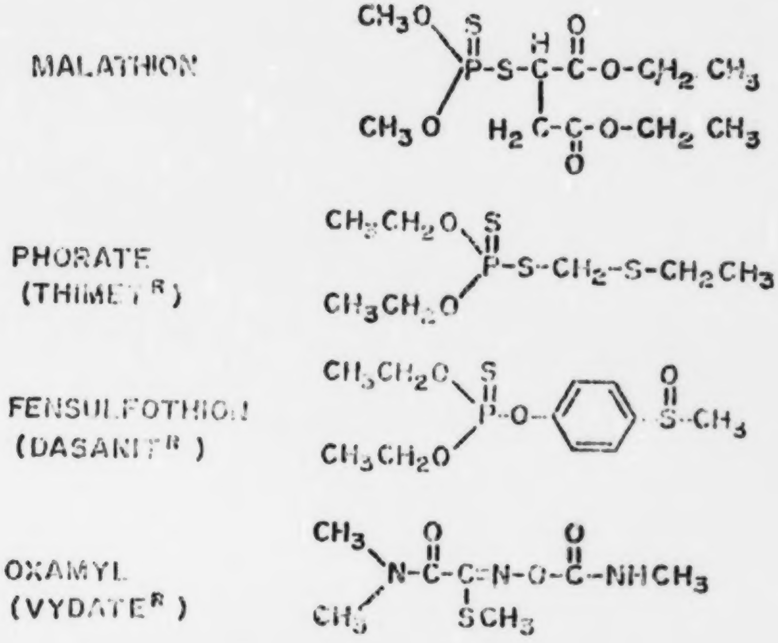


Figure 6



the capabilities of this apparatus. Figure 7 is a summary of the operations carried out in low-pressure liquid chromatography on silica gel using stepwise gradient elution with increasingly polar solvents. Figure 8 is a composite of a number of runs showing the responses obtained for standard phorate and its 5 metabolites using the stepwise elution shown in the preceding figure. Figure 9 gives a comparison, step by step, of analysis by conventional GC and analysis by cleanup and direct detection. Figure 10 shows a liquid chromatogram of standard solutions of Dasanit and its metabolites and Figure 11 shows the chromatogram of an extract of 10 grams of soil fortified with the same concentrations of Dasanit and its metabolites. Figure 12 presents the steps in the liquid chromatographic analysis of oxamyl in alfalfa showing first the oxamyl standard peak, second the extract of check alfalfa, and third the extract of field-treated alfalfa equivalent to a 10-gram sample. Note that these liquid chromatograms are aligned according to the time at which the acetone elution was started and not

Figure 7

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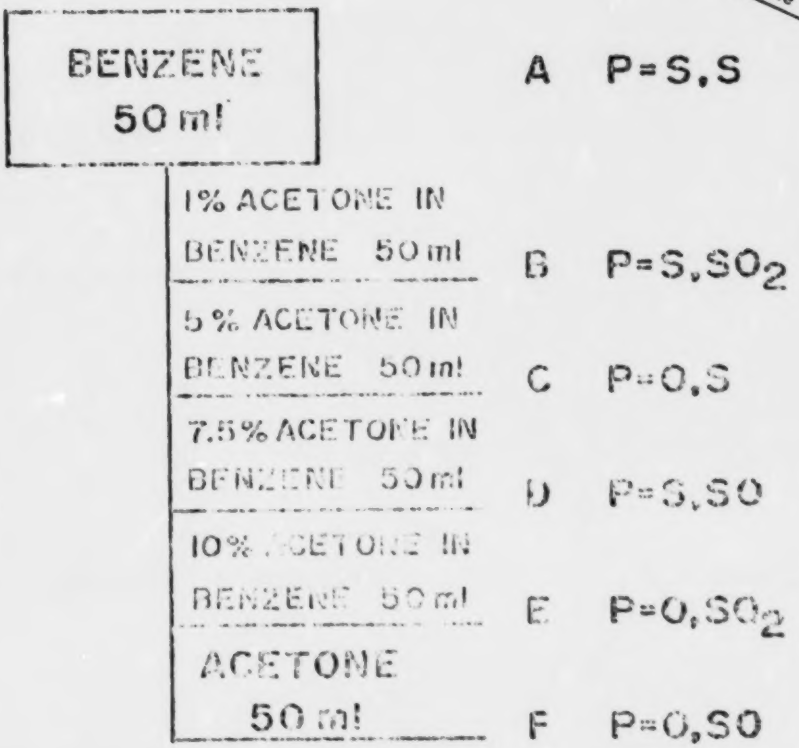
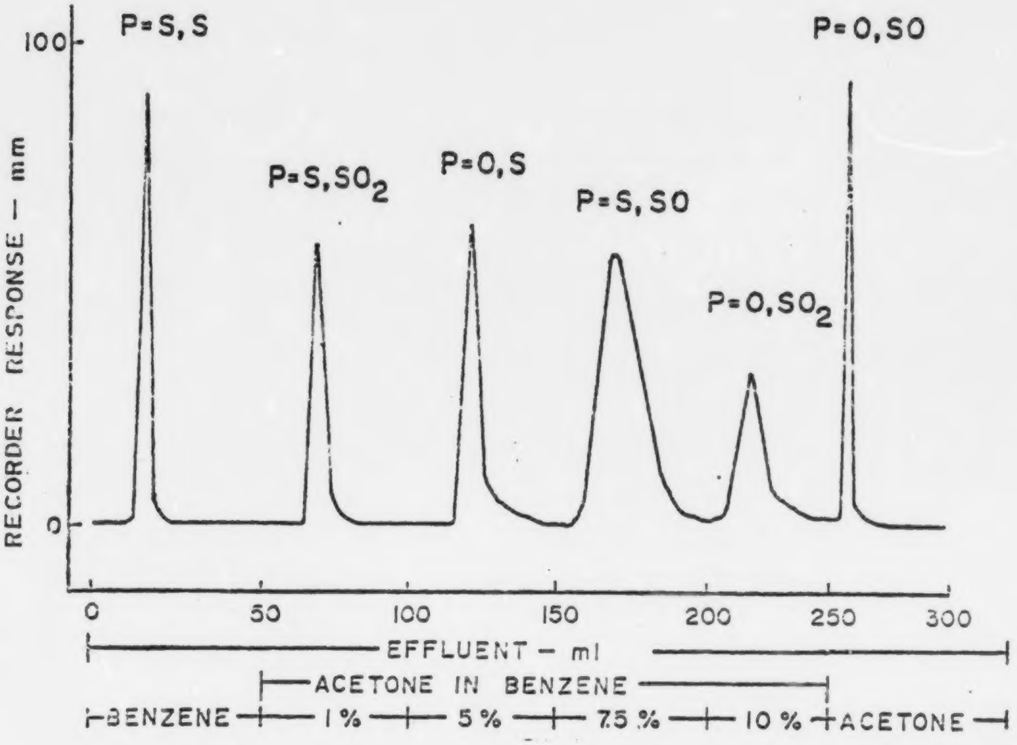

BENZENE 50 ml
A P=S,S
1% ACETONE IN BENZENE 50 ml
B P=S,SO2
5% ACETONE IN BENZENE 50 ml
C P=O,S
7.5% ACETONE IN BENZENE 50 ml
D P=S,SO
10% ACETONE IN BENZENE 50 ml
E P=O,SO2
ACETONE 50 ml
F P=O,SO

according to the injection time of the sample. This latter example was run in the sulfur selective mode. Figure 13 shows chromatograms of other pesticides to illustrate the response to various sulfur, phosphorus or phosphorus- and sulfur-containing compounds such as busulfan, coumaphos, and parathion.

During the testing of this detector system with low-pressure cleanup type liquid chromatography columns, it became obvious that the combination of cleanup column and selective detector provided a new means of rapid screening for pesticide residues. Excellent separation of pesticides and their metabolites was achieved by stepwise gradient elution and the extreme selectivity of the flame photometric detector for P and S prevented interferences from most nonpesticidal components. The major drawback was the need to replace the silica gel column for each analysis.

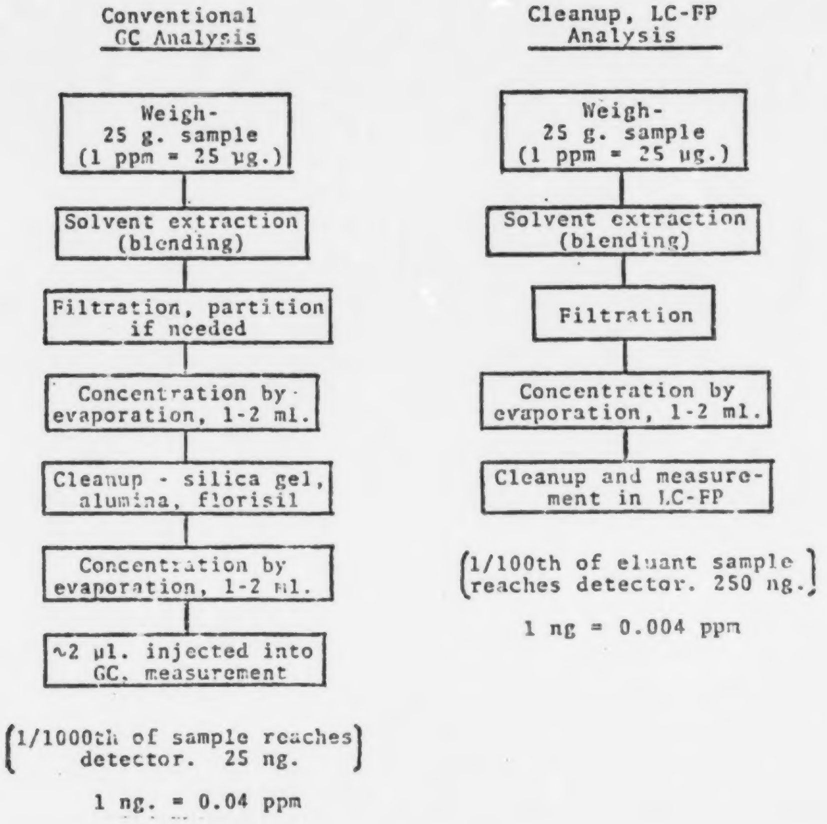
Figure 8



The possible solution to this problem came quite by accident about 9 months ago when it was discovered that EM silica gel SI-200A (mean pore diameter of 180 angstroms) which is commonly used in aqueous gel permeation work could be programmed with organic solvents to achieve almost any desired degree of separation of pesticides (3). Impurities in organic extracts such as fats, waxes, and pigments were retained by absorption near the top of the column but could be completely removed after analysis by elution with pure acetone or methanol. Such behavior is just the opposite of what is normal for gel permeation cleanup based on molecular exclusion.

In this project we will combine the Autoprep 1001 GPC cleanup apparatus with the liquid chromatography flame photometric detector to devise a semi-automatic residue analysis system for OP pesticides. Since only about 1 percent of each sample reaches the

Figure 9


Conventional GC Analysis
Weigh- 25 g. sample (1 ppm = 25 µg.)
Solvent extraction (blending)
Filtration, partition if needed
Concentration by evaporation, 1-2 ml.
Cleanup - silica gel, alumina, florisil
Concentration by evaporation, 1-2 ml.
~2 µl. injected into GC, measurement
[1/1000th of sample reaches detector. 25 ng.]
1 ng. = 0.04 ppm
Cleanup, LC-FP Analysis
Weigh- 25 g. sample (1 ppm = 25 µg.)
Solvent extraction (blending)
Filtration
Concentration by evaporation, 1-2 ml.
Cleanup and measurement in LC-FP
[1/100th of eluant sample reaches detector. 250 ng.]
1 ng = 0.004 ppm

detector, the remaining fraction can be collected for analysis of organochlorine pesticides by GC, nitrogen-containing pesticides by GC or TLC, and the confirmation of the OP pesticides by GC-MS, if needed or desired. The research is divided broadly into three major phases. First, combine the Autoprep 1001 and the LCFP detector and investigate the performance of the combination for cleanup and screening analysis of 5 P- or S-containing pesticides selected from the priority list, using 5 substrates as decribed later. Second, determine the performance parameters and capabilities of the SI-200A silica gel as a reusable cleanup and analytical column packing in a low-pressure chromatograph system employing the same pesticides and substrates as in Part I. Third, if the results of tests under Parts I and II are satisfactory,

Figure 10

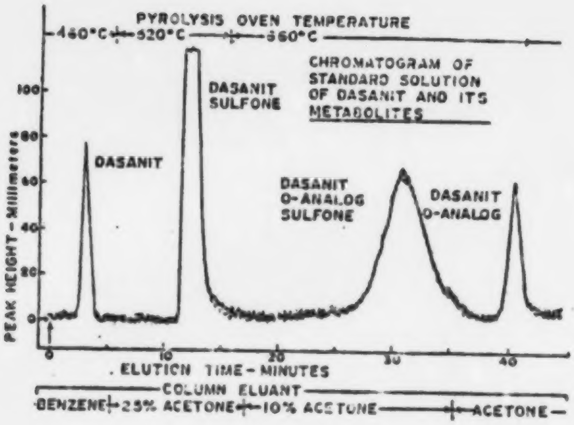
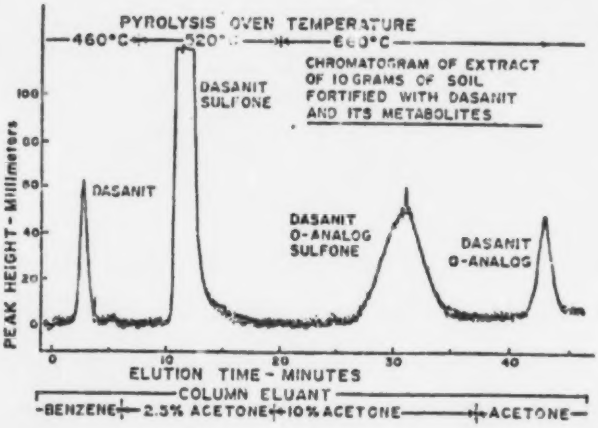
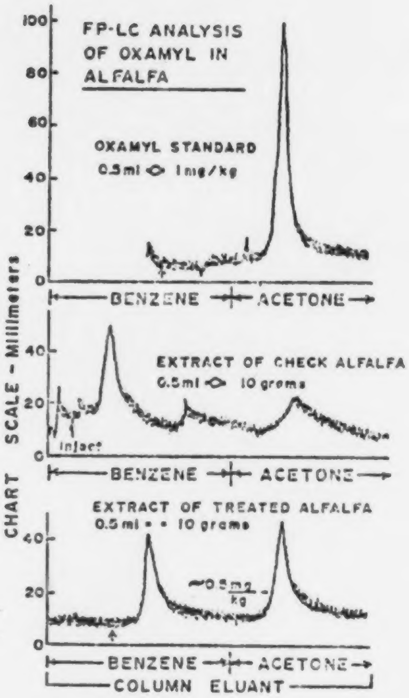


Figure 11



combine I and II to create a semi-automatic residue analysis system and investigate and define its optimum operating conditions. Testing will be confined to the following pesticides: aldicarb, phorate, captan, demeton, and endosulfan. All significant metabolites (the oxygen-analogs, sulfoxides, sulfones, etc.) of these compounds will be included. Although not on the substitute chemicals list, chlorpyrifos (Dursban) will be included as a convenient reference compound since it

Figure 12



contains chlorine, nitrogen, phosphorus, and sulfur. The substrates that will be used for testing cleanup efficiency are animal fat, milk, soil (Beltsville sandy loam), alfalfa, and one vegetable or fruit selected from the group of tomatoes, carrots, and apples. All work will be carried out using fortified samples except for some check analyses of field-incurred residues of phorate in soil and alfalfa which is undergoing testing this year at Beltsville.

While waiting for the necessary apparatus to arrive, we have continued to modify and improve the Pye-Unicam flame photometric system by substituting stainless steel pyrolysis oven tubing in place of the silica glass tubing. This eliminates a fragile (and frequently broken) part of the apparatus and also permits improved heat control over the line leading from the pyrolysis tube to the detector.

The Autoprep 1001 cleanup system has just been obtained and we are now in the process of connecting up the LCFP detector system to it. Meanwhile, in a separate low pressure LC system, we are using phorate and its metabolites to optimize the columns and concentrations of hexane/acetone to use in the step gradient elution of SI-200A gel.

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2. Hill, K.R., and Jones, W.M. Adaptation of the flame photometric detector to liquid chromatography of pesticides. J. Chromatogr. Sci. In press.
3. Getz, M.E. A recycleable cleanup column utilizing large pore silica gel. Talanta. In press.

DR. MOSEMAN: Ken, have you done anything or have you noted any effects of water on the silica gel?

MR. HILL: No, we have not.

DR. MOYE: Would you comment on the SI-200 gel. Is that a polyacrylamide?

MR. HILL: I don't believe it is. I'm not too sure of the exact structure of it. It's probably a silica gel. It's one of a series of three gels they sell for this work. Three different exclusion ranges.

DR. MOYE: Are you limited on your solvents in terms of molecular volumes?

MR. HILL: Yes. That's why you don't see acetonitrile used in our work.

QUESTION: I was interested in your comment to the effect that you really don't care how much metal you have or what types of metal you have in the pyrolysis unit, but you're trying to combust it anyway. This reminds me of some work I did years ago where I was trying to pipe parathion through a length of stainless steel quarter-inch tubing, and it was quite surprising to me how much of that material is simply lost, irreversibly lost on surfaces. Whether there is a decomposition to inorganic phosphates or what, I don't know, but I'd be interested to hear what results you get.

MR. HILL: I didn't really have time this morning to go into some of the performance peculiarities of this system, but one thing with the old silica tubing system (the reason we said we were skeptical was to whether pyrolysis was the mechanism) was that if you start out at a low oven temperature, say 300 degrees, and you inject equal quantities of something like parathion and raise the oven temperature stepwise at 50 degree intervals, the signal starts to go up becuase the wire is going very fast, 30 centimeters a second. The signal starts to climb, and then you would expect, if pyrolysis was the mechanism, that when it reached a total pyrolysis condition that it would simply plateau out. In actual fact it starts down again. There's an optimum temperature, apparently. We assumed this meant that we had actually been increasing the rate of molecular vaporization and that when we reached a pyrolysis condition a new species was being formed which the detector couldn't see.

QUESTION: What sort of band spreading or carry-over or memory effects might you anticipate in the pyrolizer?

MR. HILL: In the pyrolizer, we're going to have to be very careful with that, we think. I have a young, new technician working on it right now. We hope we can keep him on for the entire contract. He's strictly here for the summer. He has been looking into this and apparently is finding some hangup in this steel tube system. He finds if he keeps shooting in stuff fast enough he can plateau out very quickly.

QUESTION: I would think that if you go to a system, eventually, in some way, shape, or form, a moving wire type of transfer system (transport system) would severely limit you in terms of using buffered systems possibly. I'm just hypothesizing.

MR. HILL: With the Pye-Unicam system as it's normally sold and used with a flame detector, for example, the signal is very noisy due to hydrocarbon impurities on the wire. The man who originally helped develop this with me, Bill Jones, unfortunately died of a heart attack and he had developed a skill so he could use one spool of wire with maybe ten passes -- you know, wind it all the way up and reverse it back and forth. We put a new man on it in the first week, and I think he was breaking wire about once a day.

DR. R. T. ROSS: In replacement of the conventional flame for the old FPD system, with your pyrolysis here, does this eliminate your problems with fogging of the quartz windows? Are you using the same melpar photomultiplier tubes?

MR. HILL: Yes.

DR. ROSS: Are these mounted similarly? I'm talking about the one that you see in most of the Government laboratories, mounted on a Tracor instrument.

MR. HILL: That's a standard Tracor flame photomultiplier burner housing and it is modified. I didn't want to go into the details, but the publication on this will indicate the modification consists of changing the carrier inlet from the bottom to the side. You drill a hole, tap it, and plug up the bottom.

DR. ROSS: Is it necessary to use a venting system or anything of this nature?

MR. HILL: No, essentially the solvent is being removed from the wire from the little solvent removing oven which is there, nitrogen flow plus heat. Then it goes into the pyrolysis oven at which point you should have mostly pesticide plus any other crud coming along.

DR. ROSS: Do you project increasing the number of specific items that you can detect, for example use of more than just your photomultiplier tube and so forth, trying to look at other elements?

MR. HILL: No, not in the immediate project.

DR. ROSS: Is this projected?

MR. HILL: We have not tried other applications, such as hanging a Hall detector in there, of course. Anything you can wedge in should be useful. Obviously at some time I'd like to see somebody try that with our or somebody else's system using nitrogen-selective detectors. I must point out that we're not trying to make any claim at all that this is a commercially viable system. As we have it now it's spread all over a bench; it looks like a mass spectrometer. It takes about as long to learn to operate. The Pye System was selected simply because it was available and worked reasonably well.

The other systems that were available, such as the Nuclear Chicago one, were being phased off the market because of lack of sales. We bought one of their continuous chain systems. There is no reason it shouldn't work. We hoped there would be a commercial development of something similar to this. That's the important thing. The sensitivity is essentially that of the photometric detector, and there you get a better split ratio than you achieve in normal analysis essentially. We have not achieved the sensitivity predicted and we're not at all sure why. We're not seeing as much in our flame photometric as we know we should be able to.

MULTIRESIDUE METHODOLOGY

Dr. Robert Moseman

The contract with the University of Florida deals with extension of multiresidue methodology. The principal investigator is Dr. H. Anson Moye. As Dr. Moye indicated yesterday, out of the list of approximately 35 compounds we were working with, as would be expected, we encountered some problems with a few of them. What we would like to do this year is work on solving those problems we encountered last year, and in addition to that, add a few compounds to the list, again working with soil. We had some problems with the extraction of certain pesticides from soil, and we will attempt to overcome those problems.

The cleanup system that was found last year to be quite effective was a silica gel column, and this year we will continue to pursue that avenue. We had problems with the derivatization. Last year we had limited ourselves to two reagents, the dichlorobenzene sulfonyl chloride and the pentafluoropropionic anhydride. In this year's effort we intend to look at some other derivatizing reagents.

Quantitative aspects of the efforts of this year will be limited to gas chromatography on standard GC columns using electron capture, flame photometric, and the Hall detector.

Some of the compounds that presented problems as far as the GC was concerned last year are amitrole, benomyl, monochrotophos or Azodrin, aldicarb or Temik, and diallate also known as Avadex. These compounds could not be handled intact through the GC, as would be expected if you look at the structures. We were also unsuccessful in obtaining any kind of a reasonable derivative.

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*Office of Research and Development, Pesticides and Toxic Substances Effects Laboratory, Research Triangle Park, North Carolina

These are a few of the derivatizing reagents that we would like to take a look at: the N-methyl-bis-(heptafluorobutyrl) amide will react with OH, NH_2, and NH functional groups; the nine anthracene methanol will react with the carboxylic acid. If this material is refluxed with thionyl chloride, it can be converted to nine anthracene methanol chloride, which will react with OH, NH_2, or NH. Pentafluorobenzoyl chloride will react with OH, NH_2, and NH.

The pentafluorobenzoyl hydroxylamine hydrochloride will react with carbonyl. Acidic acid -N- hydroxysuccinamide ester should react with NH_2 and the NH.

In addition to these derivatizing reagents, if we find something during the contract period or if anyone here has some suggestions, we'd like to try those also.

The compounds that we had problems with in the extraction from soil are captan, folpet, Difolatan, Zectran, carbofuran, and monuron. There were three different types of soil that were fortified at four different levels. These compounds gave us either no recovery or low recovery, and Dr. Moye, I think, indicated yesterday that possibly one other compound, IPC, also gave us some problems.

Captan, folpet, and Difolatan presented problems with recovery in that they were decomposing in the boiling methanol-benzene that was used for Soxhlet extraction. For the other compounds recovery was simply lower, and correct me if I'm wrong, Anson, but you attempted to improve the recovery of captan, folpet, and Difolatan by simply tumbling for extraction, but there again we had low recovery.

DR. MOYE: No, we actually did quite well with tumbling in benzene for just these three. The tumbling was not nearly as satisfactory with all of the other classes of compounds. But for those three we were able to get 90 to 100 percent recovery.

DR. MOSEMAN: In our effort to try to improve the extraction efficiency, what we would like to do this year is use the polytron homogenizer to extract soils. This device combines ultrasonic energy with mechanical shearing. It's a high-speed blending action and has been reported in the literature to be very good for recovery of chlorinated hydrocarbon pesticides. That is about the extent of the work that has been done there. We would like to use this apparatus to improve our recoveries for some of these compounds.

There are four organophosphates that we would like to add to the list: Diazinon is pretty widely used; chlorpyrifos or Dursban; dimethoate; and a compound of similar structure, phosphamidon.

Some additional compounds are methomyl or Lannate, which is going to be a difficult compound; and Landrin with its two isomers, the 3,4,5- and the 2,3,5-isomers; dinoseb or DNBP; and dicamba, the last two being herbicides.

These compounds will be examined in the same manner used for the other compounds. They will be fortified in soil, extracted, cleaned up on silica gel, derivatized if necessary, and carried through the standard GC quantitation.

To summarize what we have in mind: first of all, we will try extraction of soil with a polytron. Results obtained will be compared to those we got last year by either Soxhlet extraction or tumbling with benzene-methanol. We propose to fortify soils at two spiking levels, 0.01 and 0.1 parts per million. Extractions will be done in triplicate, using, to begin with, benzene-methanol since we have a handle on that, but we can also look at other solvents for extraction.

For cleanup and separation, the silica gel column system worked well last year with the five fractions. Derivatization is going to be required. As I mentioned earlier, we're going to look at some other derivatizing reagents to see if we can find a more universal derivatizing reagent to handle more compounds. Finally, quantitation by standard gas chromatographic columns and electron capture, flame photometric, and the Hall detector.

DR. ROSS: On your universal derivatization agent, do you have any speculations there on what you're going to use? I mean, do you have some idea? Certainly there should be suggestions.

DR. MOSEMAN: We want to look at those reagents that I showed, to screen them.

DR. ROSS: The acid chlorides.

DR. MOSEMAN: Yes. And I think Dr. Moye has looked into that a little more closely. Possibly the nine anthracene methanol reagent, which would cover a wider range of functional groups, may be an approach that we could use to give us broader coverage.

DR. MOYE: Really, what we hope to accomplish here, I think, is a more potent acylating reagent that will perform with the carbamate-like materials such as aldicarb and methomyl. We were not able to derivatize those two materials last year with the anhydride.

This N-methyl-bis-(heptafluorobutyrl) amide is a very potent acylating reagent. And this material has been shown to acylate compounds that have not been satisfactorily acylated with the anhydrides. So that, I would think, would be the prime prospect for those two compounds, along with all of the N-methyl carbamates.

DR. ROSS: In other words, by referring to a universal derivatization agent, you're not talking about one agent that would derivatize all compounds. It's kind of a little bit overoptimistic.

DR. MOYE: It is somewhat a little bit overoptimistic. Now, this nine chloromethyl anthracene, as Bob mentioned, is an acylating agent, if you will, that will react with many functional groups. And possibly, this reagent can be as effective for things like aldicarb and methomyl as well as for some of the materials that have other functional groups.

DR. MOYE: Yes. I think we're going to concentrate on the difficulties with the carbamates and the carbamyl oxime, that is, methomyl and then the aldicarb -- also Zectran has been a problem -- and see if we can't solve those three with the acylating agent. That will be a big step forward, I think.

QUESTION: I also gather you're switching from Florisil to silica gel. This is somewhat different from yesterday's.

DR. MOYE: No.

DR. MOYE: No. We used silica gel this past year.

DR. MOSEMAN: We can handle a lot more compounds with silica gel than we can with the Florisil. And the separation into five fractions is quite helpful. It aids in identification of the compounds. You know which fraction it's going to come in. As far as the derivatization reagents, what we may have to do -- I'm not saying we're going to work on this, but I don't think there's one reagent around that is going to derivatize everything that we're after, but it would be nice -- would be to split the cleaned up extract into subportions and use different derivatizing reagents, depending on what compounds you're looking for. That's a possibility. It becomes more and more involved as you try to handle more and more compounds with a single system.

DR. ROSS: Bob, don't you think this would gradually progress in developing these more sophisticated systems such as Dr. Hill has discussed, and this is what we're going to eventually get away from, this derivatization?

DR. MOSEMAN: Some of it, yes. I think what Dr. Hill is doing is going to complement what we're going to do very nicely. There are certainly going to be compounds we just cannot handle with standard, or what we refer to as standard, extraction, cleanup, derivatives, standard GC type approaches. We're going to have to go to something else. There's a limit to that. And the LC system is a way to go. The high-pressure LC system is the way to go, a good way to go.

DR. MOYE: We haven't abandoned derivatives. We just have a lot of projects.

PESTICIDES IN AMBIENT AIR

Dr. Robert Lewis*

Like most of the other programs you've heard about this morning, our programs which are directed toward the development of methodology for determination of pesticides in the air are just getting underway. The effort is going to be largely extramural; however, we do have a relatively small in-house involvement.

We began our extramural program last month by awarding a contract to Southwest Research Institute in San Antonio, Texas for evaluation of various collection media for low levels of pesticides in air. Dr. Donald Johnson and John Rhodes at Southwest will be responsible for this project.

I think we need a little background at this point. Several years ago one of the predecessor segments of EPA, the National Air Pollution Control Administration, had developed a high-volume air sampler for airborne pesticides under contract with Syracuse University Research Corporation. Dr. Gunter Zweig was intimately involved with that project.

Basically they modified the standard Hi-Vol air sampler, which has seen very wide use for years by EPA for collection of particulate matter from air. These samplers are used today in great numbers. If you look at Figure 1, you'd say it's a Hi-Vol sampler because it is a shelter of a Hi-Vol sampler. Inside you can see the differences. The modification essentially was the addition of the pesticide collection module at the top of the sampler. In Figure 2, you can see that it consists simply of a standard piece of glass plumbing, 4 x 2-inch reducer, affixed to the top of which is a glass fiber filter mat, same type used in the Hi-Vol. The filter is capable of collecting particulate matter down to at least three-tenths of a micrometer in diameter.

This is followed by a bed of glass beads, which are coated with a liquid collection medium for entrapment of pesticide vapors. The sampler was designed to

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Figure 1. Complete Airborne Pesticide Sampler

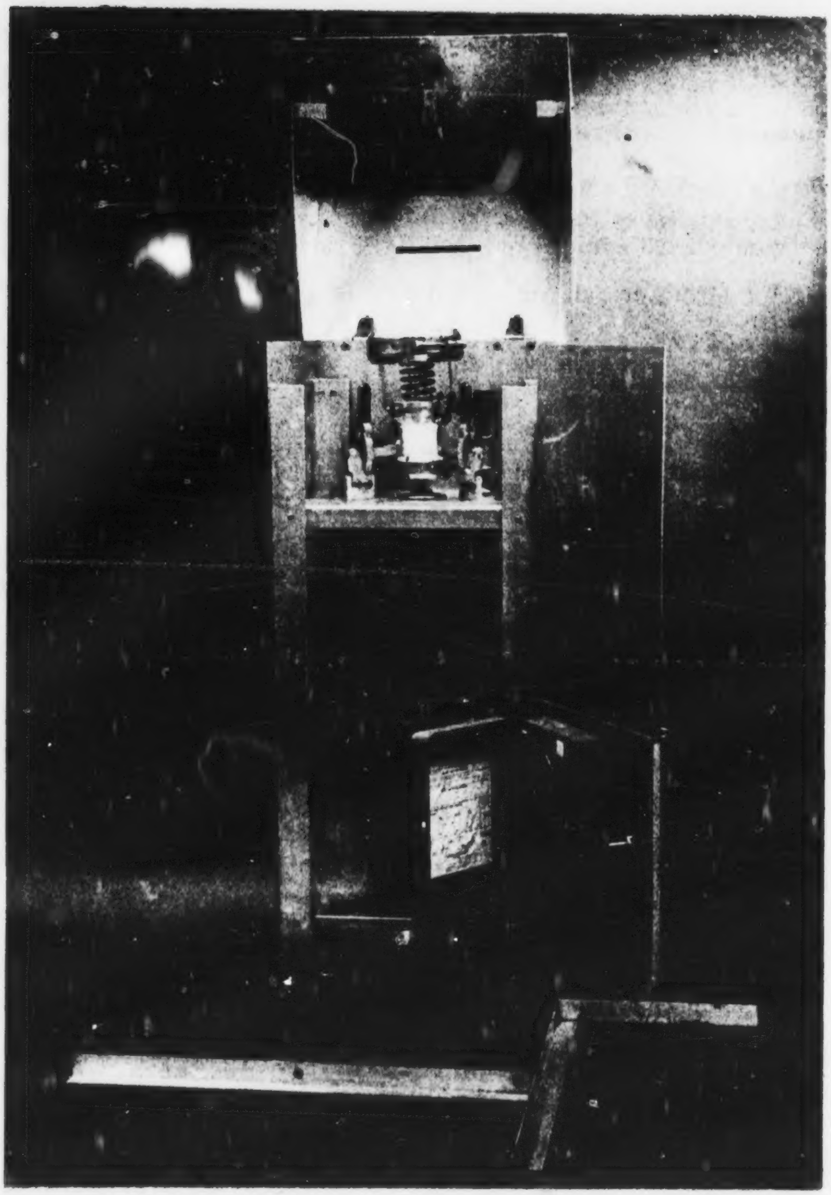
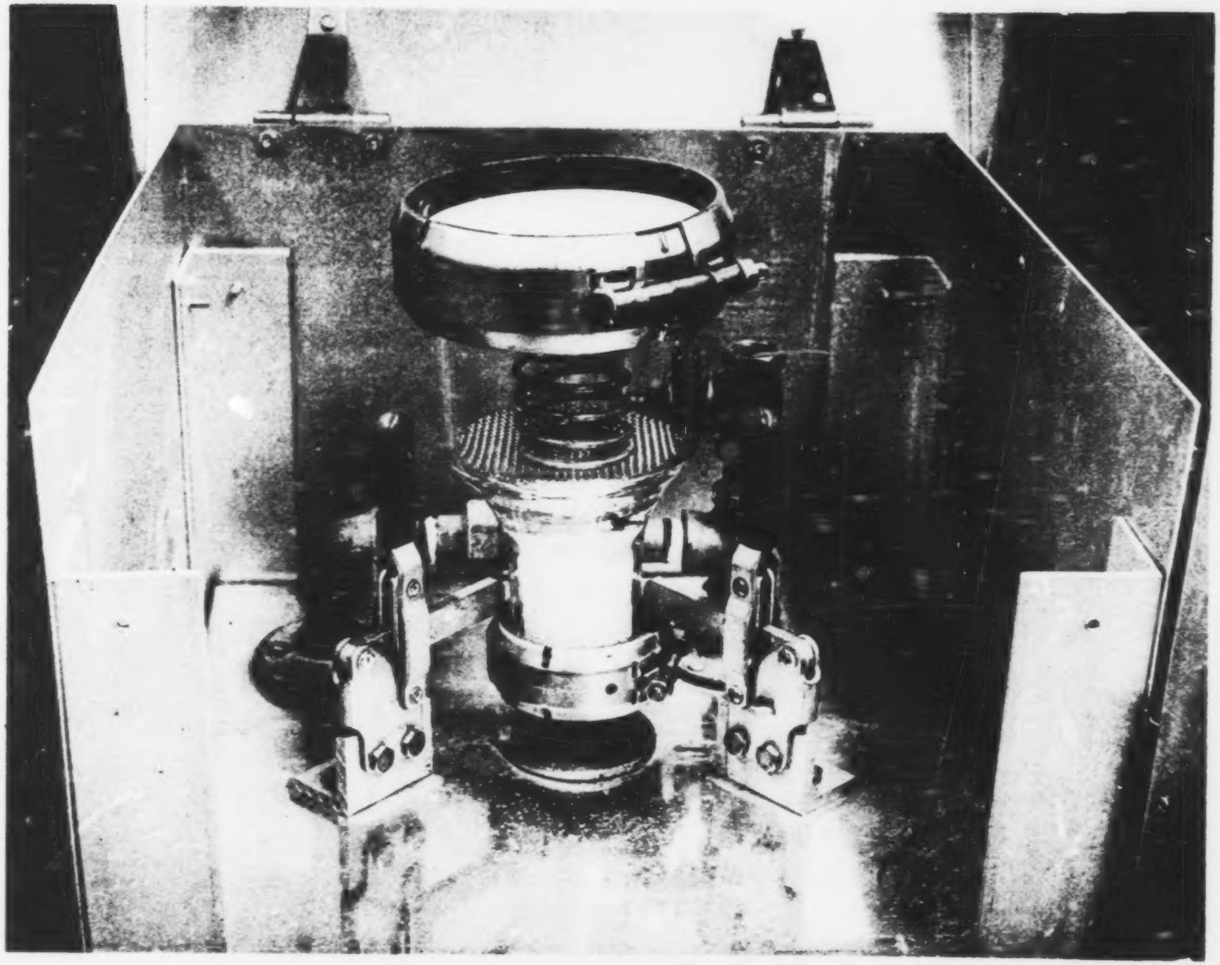


Figure 2. Pesticide Collection Module Compartment with Module Installed



operate at 280 liters per minute (10 CFM) and the contractor found that cottonseed oil was the most efficient collection medium for the sampler operating under these conditions. The collection efficiencies which they determined for cottonseed oil were indeed quite impressive.

There were, however, a number of drawbacks to the use of cottonseed oil as the collection medium for pesticides, not the least of which is the rather involved isolation in cleanup methodology necessary before pesticides can be finally determined by GLC. This is essentially the Mills-Olney-Gaither procedure for pesticides in fat. Another problem is the fact that cottonseed oil is an unsaturated material. It is readily oxidized, the extent of oxidation being very dependent on the condition of the atmosphere in which the sampling is taking place. But it is frequently oxidized and sometimes polymerized during the sampling process, which further complicates the extraction of the pesticides from it.

The sampler itself is a very good one for ambient air monitoring. It is simple in design, very easy to operate, has very few maintenance problems, and is very inexpensive. And I think present day costs for constructing one of these is only around $450.00. With all the parts we have in the Agency for Hi-Vols, it is probably less than that.

We thought it was well worthwhile, therefore, to look for substitute collection media for cottonseed oil for use in the SURC sampler; hence, the contract to Southwest.

The objectives of the contract are:

1. To evaluate other liquids as substitutes for cottonseed oil in the SURC samplers.
2. To evaluate support-bonded sorbents as collection media for airborne pesticides.
3. To modify or design collection devices for most efficient use of collection media.

We are particularly interested in gas chromatographic liquid phases. Since the sampler only uses three milliliters of liquid, this is quite feasible from an economic standpoint. We have some preliminary data generated in-house that suggest certain liquids like diethylene glycol succinate might have a lot of promise. Silicone oils will be evaluated and, of course, we're going to examine a number of others.

The second objective is to look at solid sorbents as substitutes for the cottonseed oil, such things as support-bonded LC packings, Tenax, macroreticular solids, such as Chromasorb-101 and -102, and polyurethane foam, which Bittleman and Olney have found to be quite efficient for collection of PCBs and chlorinated insecticides.

The final objective is to modify or redesign the sampler as required to accommodate the new collection media. We do not anticipate that much modification will be required. The sampler seems to be well adapted to using a variety of types of collection media. We know it operates well with polyurethane foam, for example.

The properties of liquid collection media that will be required are:

1. Proper viscosity to coat glass beads
2. Sufficiently low volatility
3. Melting point above 20° F
4. Chemical stability
5. High absorptivity for wide ranges of organic compounds
6. Chemical inertness
7. Acceptable recoveries of pesticides
8. Amenability to routine pesticide separation and cleanup, including new methodologies.

These will be used as the criteria for the selection of candidate media. The first three criteria apply exclusively to liquids, the last five apply to solids as well as liquids. We are not looking for new analytical methodology development, but for procedures that are amenable to current analytical methodologies.

Ideally we would like to minimize the number of steps between the extraction of pesticide from the collection media and its determination by gas chromatography.

The following is the list of study pesticides which Southwest has proposed to work with. I think it is an excellent list for the study.

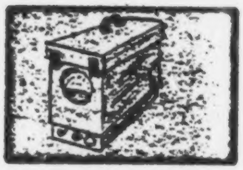
Chlorinated	Phosphates	Carbamates
Alpha-BHC	Phosdrin	Carbaryl
Lindane	Diazinon	Carbofuran
Aldrin	Methyl parathion	
p,p'-DDE		
p,p'-DDT		
Mirex		

We did not propose the study pesticides. Not all of them are substitute chemicals. But all three important classes of pesticides are covered, along with a wide range of volatility and polarity within the groups. Methods will have to be developed for generation of test atmospheres of these pesticides for introduction into the sampler to determine collection efficiencies. The method that Southwest has proposed will work quite well. It's somewhat novel and I will not elaborate on it.

The sampler will also be field tested for a period of at least one month. Of 13 respondents to our RFP, Southwest was unique in proposing the generation of test atmospheres of pesticides in the field during field testing, as well as testing the sampler in the ambient air pool. I think that sums up pretty well what our extramural program is going to involve.

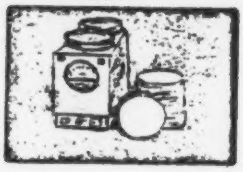
I want to mention briefly one of two in-house projects that we have, that is, evaluation of the sampler shown in Figure 3, which is manufactured by the Environmental Research Corporation, St. Paul, Minnesota. It is a small, portable air sampler, designed primarily for collection of organophosphorus compounds and for short sampling periods. We view it as a "source-type" sampler, perhaps of some value in sampling air in recently treated fields for the purpose of reentry assessment.

Figure 3



The current price for the unit is $1500.00, which appears to be inflated. If the lid, which is also the rain cover, is removed (Figure 4) with care not to break the plastic hinges, you can see that it has two assemblies for holding filters. Air is drawn through both filters simultaneously. The filters consist of Poropack R sandwiched between two surfaces of glass fiber filter. They also are quite expensive, costing $20.00 a piece. You are looking at $1,000.00 worth in Figure 4. The sampler uses two at one time and they are not reusable.

Figure 4



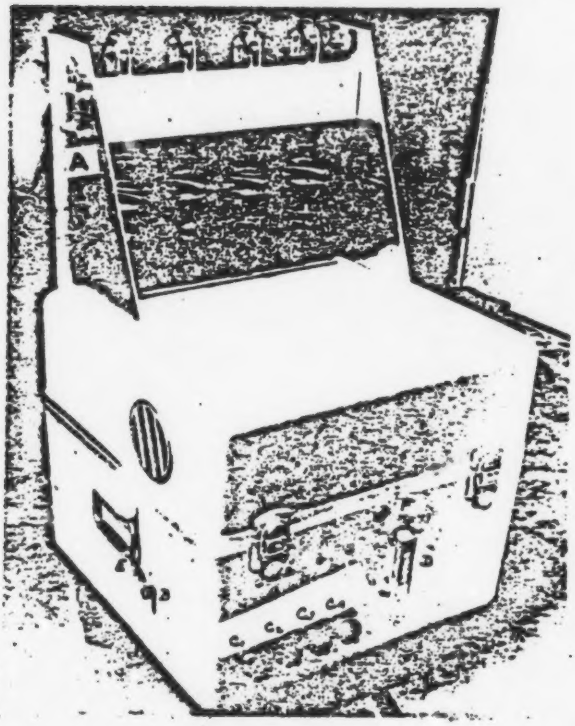
This particular model has two vacuum-cleaner motors (Figure 5); one for AC, one for DC current. Of course, only one is operated at a time. Air is pulled through both filters by one motor. To convert from AC to DC, you simply rotate the top portion 180 degrees so that the tubes which lead to the filter housing fall into the proper sockets. The sampler can operate at rates up to about 170 liters per minute. It is said by the manufacturer to be efficient for the collection of malathion for periods up to about 3 hours.

Figure 5

We are currently evaluating the sampler. Work started yesterday, after rains delayed the project in experimental tobacco plots which have been treated with methyl parathion, both in the microencapsulated and emulsifiable concentrate forms. The work is being conducted as part of a cooperative effort with investigators from North Carolina State University who are going to be looking at dislodgeable residues at the same time.

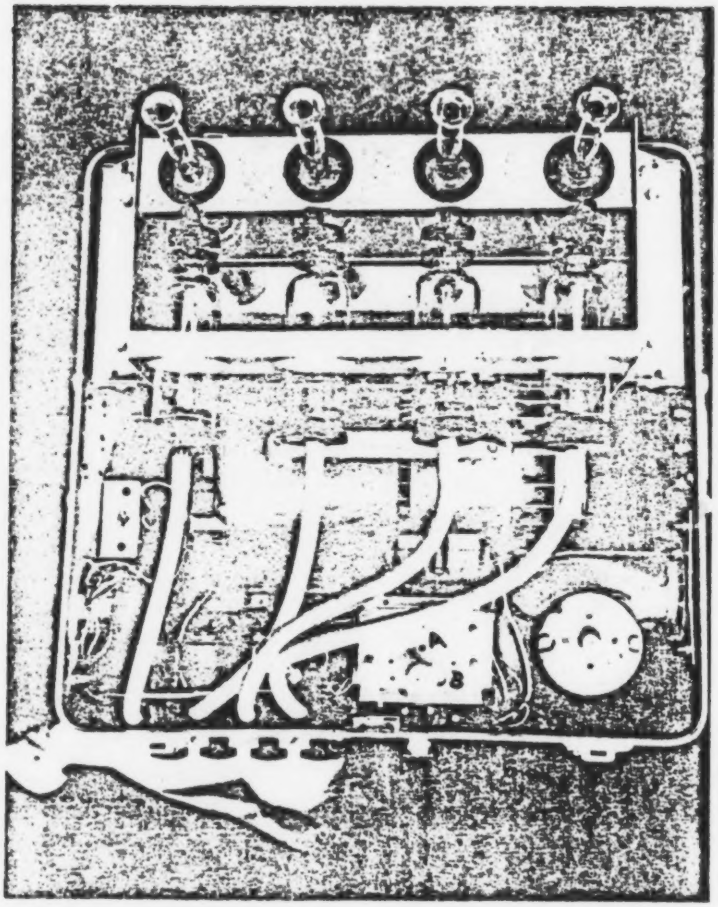
At the same time, we are comparing the sampler with the SURC sampler and with the MRI or MISCO sampler (Figure 6). There may be some people here who are not familiar with the MRI sampler. This is a sampler that was developed some years ago by Miles, who was with the Public Health Service, was further developed by Midwest Research Institute, and is now available from Microchemical Specialties Company (MISCO). It was used by EPA's Division of Pesticide Community Studies for 3 years in a rather extensive network throughout the United States to collect pesticides from the air. Dr. Anne Yobs was primarily responsible for the project. The MRI sampler has, therefore, seen more use than any other sampler for sampling airborne pesticides, and is considered to be more or less the industry standard. So we will be comparing all of our results to those obtained with the MRI sampler.

Figure 6



The MRI sampler uses impingers charged with ethylene glycol. The clockwork shown on the inside (Figure 7) is pretty well inaccessible while the sampler is operating. Some of the features relate to its unsuitability, in my opinion, as a sampler for ambient air. It is a low-volume air sampler, with a maximum sampling rate of about 20 liters per minute. It is also expensive. I imagine that the price is nearly $2,000.00 now. This one cost over $1,600.00 about 3 to 4 years ago. It is complicated to operate, bulky and heavy. The impingers are fragile and require a certain amount of skill to load and quantitatively transfer the collection medium to other vessels prior to analysis. We're using the MRI sampler only because most of the air data that are available were collected with this sampler.

Figure 7



DR. ZWEIG: Bob, I think it is rather important to also determine in what state the pesticides exist in ambient air. I remember when I was connected with the project at Syracuse University several years ago, we were trying to distinguish between pesticides and the vapor phase as an aerosol, and absorbed on particulate matter. I would think that in your present studies these two questions could be answered.

If you extracted the filter, which we never did, this would give you an idea of pesticides on particulate matter that are caught in the filter. As far as vapors and aerosols, this I think is indistinguishable from the liquid or even solid media. I think strictly from a basic point of view and also from a toxicological point of view, it is extremely important to find out in what shape and form pesticides exist in ambient air.

DR. LEWIS: I agree with you 100 percent on that point. But it is not so simple as you describe. We have looked at what is on the particulate matter and we find very little there. When we have taken these filters which already had particulate matter collected on them, and spiked them with a wide variety of pesticides (at least 20), then drawn air through the filters for another 24 hours, we find that all of the pesticides which we put on were stripped off (even DDT, to the extent of 95 percent). So a sampler designed to distinguish between pesticides in the particulate-absorbed state and the vapor state is going to have to have a different design than this one. You have to get the particulate matter, once it is collected, out of the air stream and that is going to involve a fairly complicated sampler. We have, in fact, in our objective statements for this fiscal year, proposed a contract to answer this question; whether or not we will get funds for it is very doubtful right now.

DR. ROSS: One comment, and a question. First of all the comment. I was talking with Midwest Research Institute not long ago, and they are still using a modified version of the MRI collector which is extremely compact. Now how expensive it is I don't know, but somewhere in my files I can look up the person to get in contact with. I think it might be worth consulting these people because they are still using this for measuring OP compounds around fill areas and so forth.

DR. LEWIS: But as I say, we're looking primarily in this contract for ambient air monitoring involving low levels of pesticides. The smaller you go in impinger systems, the more you're limited on the amount of air you can sample in a given period of time.

DR. ROSS: Dr. Zweig, do you know anything about this system that Midwest Research Institute is using? I forget the person's name momentarily.

DR. ZWEIG: No, all the people who were connected with the original project about six years ago have left.

DR. ROSS: Well, this person has modified it. As a matter of fact, he had his first two systems working when I was there. It is very, very small and I've forgotten the flow rate. You see, it was very high and very effective.

DR. LEWIS: It's too small.

DR. ROSS: That's not what he was explaining to me. I can give you specific--

DR. LEWIS: You can't get the flow rate needed for ambient air monitoring through such a small system.

DR. ROSS: Then my question was back to using solids, you mentioned ethylene glycol.

DR. LEWIS: No, diethylene glycol succinate.

DR. ROSS: Are you going to have to go through the long quality control extractions and everything as was done some years ago? Do you have to do this?

DR. LEWIS: No, we found that using unstabilized DEGS, we could extract pesticide from DEGS and inject it directly into GC with very little or no background problems. However, you probably would have to use at least a minimal cleanup because you are going to be collecting a lot of other compounds from ambient air in addition to pesticides.

DR. ROSS: So this will not have to be incorporated into major quality control programs to accompany this particular study, right? There won't have to be a big quality control program going on simultaneously?

DR. LEWIS: I should think not. If we instituted an air sampling network, then a quality control program would be necessary, however.

DR. ROSS: You mean you're not going to do that?

DR. LEWIS: That's down the road a bit.

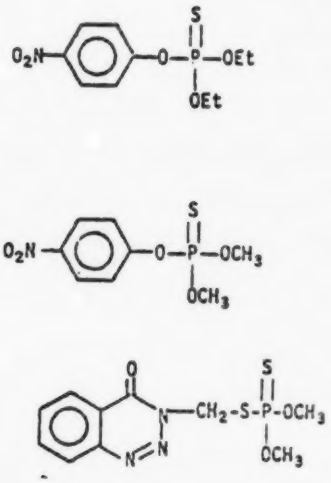
IN SITU METHOD FOR ORGANOPHOSPHATE INSECTICIDES

Dr. William T. Colwell*

The goal of this new program is the development of a simple, rapid, on-site method for the determination of residual organophosphate pesticides. Most specifically and practically the technique is designed to provide a field worker-usable process for determining safe reentry levels of organophosphates.

Figure 1 shows the three pesticides, parathion, methyl parathion, and Guthion, that will be test compounds for the feasibility study. As will be shown later, the envisioned process depends on nucleophilic attack of the test reagent on phosphorus. Base catalyzed hydrolysis of Guthion has been reported to occur primarily on the methylene carbon. Thus, it is possible that organophosphate pesticides of this class will prove refractive. If a usable portion of nucleophilic attack occurs on phosphorus or if the methylene moiety is sufficiently reactive for the ensuing alpha-elimination reaction, then the process could prove general.

Figure 1



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*Stanford Research Institute, Menlo Park, California

A general schematic of the first process to be investigated is shown in Figure 2. The dectector reagent, or a reactive salt, is allowed to react with the pesticide to produce an odoriferous compound, in this case methyl isonitrile, and detection is by smell. Several points should be noted that are relevant to the rationale of the research:

1. A sample area of the field crop or a collection of leaves, etc., in a container is all that needs be used. Stated another way, only aliquots of the field are involved and the test material can be discarded.

2. The only equipment required is the preformed detector reagent and a field worker or workers of known sensitivity to the isonitrile.

3. Methyl isonitrile is an extremely potent and characteristic odorant. In previous work we had shown that the detection and recognition thresholds of the compound are about 10^{-8} g/liter of air. Alternatively, fairly sensitive copper-benzidine air sampling tubes can be used for visual isonitrile detection.

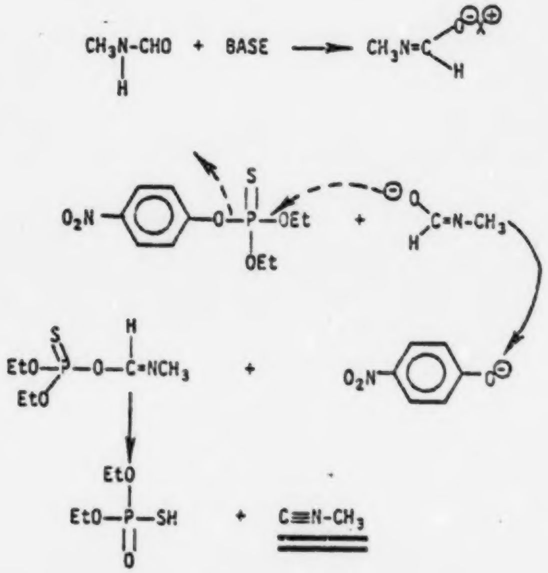
Figure 2

$$\begin{array}{c}\text{Detector}\\ \text{Reagent}\\ (CH_3NHCHO)\end{array} + \begin{array}{c}\text{Organic}\\ \text{Phosphate}\\ \text{Pesticide}\end{array} \xrightarrow[\text{reaction}]{\text{rapid}} \begin{array}{c}\text{Volatile}\\ \text{Compound}\\ (CH_3NC)\end{array} \longrightarrow \begin{array}{c}\text{Subjective}\\ \text{Sensing}\\ \text{(Odor)}\end{array}$$

These points can be amplified upon. When finally developed, the method will be capable of giving a fairly absolute measurement of remaining pesticide, as a stoichiometric relationship exists between reagent and organophosphate. Only a small crop sample is used and the test odorant can be totally contained or confined to a small area. The test material and reaction product will not contact useful crop, and additional toxicity testing and registration requirements will be avoided.

There are several possible odorants that can be tested, and reagents other than methyl formamide. Figure 3 shows the details of this system, and it will be the subject of our initial work. A methyl formamide salt is generated that presumably attacks the phosphate phosphorus. The best leaving group, para-nitrophenolate, is eliminated, giving a characteristic red-yellow color. The remaining fragment undergoes an alpha-elimination reaction, well-known in isocyanide chemistry, and the odorant, methyl isonitrile, is released.

Figure 3



We have demonstrated that the reaction does, in fact, occur when we use a system that is not suitable for the simple field work detection system. A fresh solution of formamide salt was prepared from the reaction of sodium hydride dispersion and methyl formamide. A drop of parathion was added to the solution and the characteristic color of para-nitrophenolate rapidly appeared along with the readily detected odor of methyl isonitrile. The same reaction has been accomplished on a glass slide lightly coated with parathion.

The sodium salt preparation is impractical due to its hydrolytic instability. An obvious requirement for field use is the availability of a reasonably harmless, stable preparation that is sufficiently reactive to generate the odorant at desired pesticide concentrations.

Figure 4 shows some other approaches we have taken to this problem. Methyl formamide and tosyl chloride spontaneously generate product. Reactions involving these components thus serve only as negative blanks. In work involving the more reactive phosphonofluoridates and amidates, we were able to generate methyl isonitrile using zinc chloride, bromide, and iodide complexes. Parathion, however, proved insufficiently reactive. We have not yet investigated the reaction in the presence of solvents such as hexamethylphosphoramide. The ferric and aluminum chloride complexes also proved unreactive in this system.

Figure 4: Effects of Some Lewis Acid Catalyst
on the Generation of Methyl Isonitrile

(1) $CH_3NHCHO + TosCl \longrightarrow CH_3NC$

(2) $CH_3NHCHO + ZnCl_2 + TosCl \longrightarrow CH_3NC$

(3) $CH_3NHCHO + ZnCl_2 + \text{parathion} \longrightarrow$ no detectable reaction

(4) $CH_3NHCHO + FeCl_3\text{-}6H_2O + TosCl \longrightarrow CH_3NC$

(5) $CH_3NHCHO + FeCl_3\text{-}6H_2O + \text{parathion} \longrightarrow$ no detectable react

(6) $CH_3NHCHO + AlCl_3 + TosCl \longrightarrow CH_3NC$

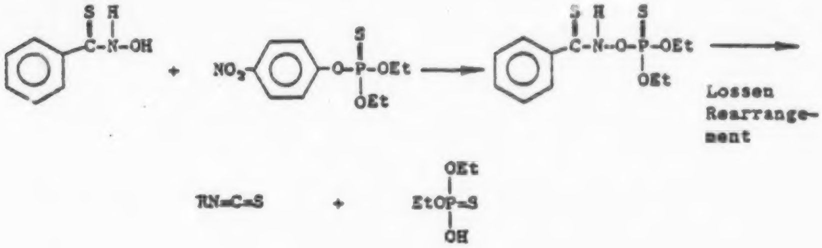
(7) $CH_3NHCHO + AlCl_3 + \text{parathion} \longrightarrow$ no detectable reaction

Other more stable formamide salts will be investigated, such as the silver and calcium salts. Also, as mentioned previously, solvent effects will be tested. Currently we have received some encouragement from preliminary reactions using ammonium bases related to those used in phase-transfer reactions. Reactions of 40 percent methanol solutions of Triton B and its methoxide analog with parathion and formamide have given the yellow-red phenolate color, but questionable generation of isonitrile. We are currently preparing hydrocarbon solutions of these fat-soluble ammonium hydroxide salts for testing. An enclosed container of hydrocarbon, methyl formamide, and the ammonium hydroxide should be hydrolytically stable and suitable for aerosol application.

Other compounds that will be tested are thioformamide salts. The thio-compounds are more nucleophilic and we hope that stable salts of these compounds may prove usable if the oxy-compounds fail.

Our in-house experience with isonitriles prejudices us to this choice of odorant; however, easily detected isothiocyanates are generated from the reaction of thiohydroxamic acids and phosphonofluoridates. This reaction will be investigated for the case of the phosphate pesticides (Figure 5).

Figure 5. Generation of Isothiocyanates from Parathion



C-N-OH
NO2
O-P-OEt
OEt
C-N-O-P-OEt
OEt
Lossen Rearrangement
RN=C=S
EtOP=S
OEt
OH

A program will be carried on concurrently with the chemical work to determine the sensitivity of a population of field hands to known concentrations of isonitrile and to determine their ability to discriminate this particular odor.

TOXIC POTENTIATORS AS BY-PRODUCTS OF ORGANOPHOSPHORUS INSECTICIDES

Dr. R. Fukuto*

Enhancement of the activity of insecticides and other biologically active compounds by other chemicals is a well-known phenomenon, usually referred to as synergism or potentiation. In the case of insecticides, synergists have been used for many years to increase the effectiveness of these materials in controlling obnoxious insects. A classical case of the exploitation of synergists in promoting insecticidal efficacy is the use of different methylenedioxyphenyl derivatives, e.g., piperonyl butoxide, sesamex, and related compounds, with the expensive, naturally occurring pyrethrins and some of their synthetic analogs.

While synergism of insecticides by the use of additives is, at least in principle, a useful idea, the same phenomenon in warm-blooded animals is obviously a matter of considerable concern. Numerous examples are known where enhancement of toxicity has been observed when a combination of chemicals is administered to a mammal. This phenomenon has been termed potentiation. Perhaps the first example of this is the potentiation of the mammalian toxicity of malathion, a "safe" insecticide, by EPN, which was observed over 20 years ago and reported by Frawley in 1957. Numerous other examples of enhanced toxicity have subsequently been reported, e.g., potentiation between Delnav and malathion, trichlorfon and malathion, trichlorfon and azinphos-methyl, and EPN and dimethoate.

Although the examples cited above consider only cases of potentiation between insecticidal chemicals, minor amounts of contaminants commonly present in technical insecticides also have been observed to produce enhanced mammalian toxicity of certain organophosphorus insecticides. An early example of this was reported by Casida and Sanderson in 1963 from a study of a technical sample of dimethoate obtained from Montecatini. The acute rat oral LD_{50} of technical dimethoate varied from 215

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*University of California, Riverside. Presented by Dr. Gunter Zweig

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to 350 mg/kg while highly purified material gave values of 550 and 650 mg/kg to the male and female rat, respectively. Analysis of technical dimethoate showed that potentiation was attributable to a petroleum ether insoluble contaminant which, when added to purified dimethoate, caused a two-fold increase in toxicity at a concentration of 10 percent (w/w). Analysis of a typical sample of technical dimethoate showed that it contained approximately 17 percent of the petroleum ether insoluble contaminant. Based on its physical and chemical properties, its impurity was believed to be related structurally to O-demethylated dimethoate. This was supported further by the finding that treatment of purified dimethoate with a demethylating agent such as O, O-dimethyl phosphorodithioic acid gave a petroleum ether insoluble product which showed potentiating action similar to that of the contaminant isolated from technical dimethoate. The structure of this contaminant, however, was not established.

A more recent report by Pellegrini and Santi (1972) describes the potentiating activity of a variety of simple trialkyl phosphorothioate and phosphorodithioate esters on the mammalian toxicity of such safe organophosphorus insecticides as phenthoate (O, O-dimethyl S-(α-ethoxycarbonylbenzyl)-phosphorodithioate) and malathion. Table 1 presents data relating to the active ingredient content and rat oral toxicity of different technical and purified samples of phenthoate, malathion, and M-1703. M-1703 is O, O-dimethyl S-(α-isopropoxycarbonylbenzyl)-phosphorodithioate and differs from phenthoate only by the substitution of an ethyl by an isopropyl moiety.

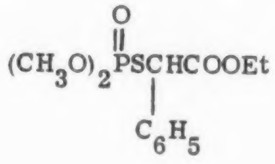
Table 1: Toxicity of Technical and Purified Phenthoate, Malathion, and M-1703 to the Rat

Compound	% Active Ingredient	Rat Oral LD_{50} (mg/kg)
Phenthoate, technical 1	61.2	77.7
Phenthoate, technical 2	78.7	118
Phenthoate, technical 3	90.5	242.5
Phenthoate, purified	98.5	4728
Malathion, technical	92.2	1580
Malathion, purified	98.2	8000
M-1703, technical	83.2	205
M-1703, purified	98	2750

The data reveal that substantial differences exist in the rat oral LD_{50} values between technical and purified samples. The largest difference was between phenthoate technical sample 1 with an LD_{50} of 77.7 mg/kg and purified phenthoate with an LD_{50} of 4728 mg/kg, a difference of over 60-fold. Tests against four different species of insects showed little, if any, variation between technical and purified samples.

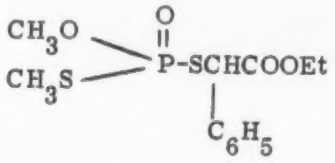
Analysis by GLC and TLC of phenthoate revealed the presence of at least six impurities. The structures of these impurities are presented in Figure 1. Analogous impurities also were present in malathion and M-1703.

Figure 1



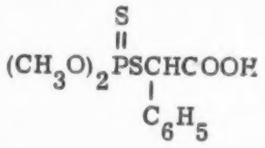
$(\mathrm{CH_3O})_2\overset{\mathrm{O}}{\overset{\|}{\mathrm{P}}}\mathrm{S}\underset{\mathrm{C_6H_5}}{\underset{|}{\mathrm{C}}}\mathrm{HCOOEt}$

phenthoate oxon



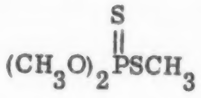
$\begin{matrix}\mathrm{CH_3O}\\ \mathrm{CH_3S}\end{matrix}>\overset{\mathrm{O}}{\overset{\|}{\mathrm{P}}}\mathrm{-S}\underset{\mathrm{C_6H_5}}{\underset{|}{\mathrm{C}}}\mathrm{HCOOEt}$

S-methyl phenthoate



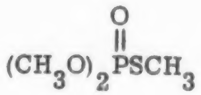
$(\mathrm{CH_3O})_2\overset{\mathrm{S}}{\overset{\|}{\mathrm{P}}}\mathrm{S}\underset{\mathrm{C_6H_5}}{\underset{|}{\mathrm{C}}}\mathrm{HCOOH}$

phenthoate acid



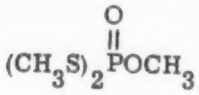
$(\mathrm{CH_3O})_2\overset{\mathrm{S}}{\overset{\|}{\mathrm{P}}}\mathrm{SCH_3}$

O,O,S-trimethyl phosphorodithioate



$(\mathrm{CH_3O})_2\overset{\mathrm{O}}{\overset{\|}{\mathrm{P}}}\mathrm{SCH_3}$

O,O,S-trimethyl
phosphorothioate



$(\mathrm{CH_3S})_2\overset{\mathrm{O}}{\overset{\|}{\mathrm{P}}}\mathrm{OCH_3}$

O,S,S-trimethyl
phosphorodithioate

TLC and GLC analysis of phenthoate technical sample 3 (90.5 percent active ingredient) gave the percentage of each impurity as indicated in Table 2. The impurity detected in largest amount was O,O,S-trimethyl phosphorodithioate which was present at a level of about 2 percent. Of considerable interest is the moderately high mammalian toxicity produced by some of the impurities compared to phenthoate itself. For example, the rat oral LD_{50}'s of O,O,S-trimethyl phosphorothioate and O,S,S-trimethyl phosphorodithioate were 47 and 96 mg/kg, respectively. This is quite surprising since neither of these compounds is believed to be a strong anticholinesterase. A study of the mode of action of these compounds would be of considerable interest since they have, until recently, not been regarded as toxic materials.

Table 2: Percent Impurities in Technical Phenthoate

	% Impurity		
Impurity	by TLC	by GLC	Rat Oral LD_{50} of Impurity
phenthoate oxon	0.03	0.024	63
S-Me phenthoate	0.4	0.32	250
phenthoate acid	1	-	4500
$(CH_3O)_2P(S)SCH_3$	2.25	2.01	450
$(CH_3O)_2P(O)SCH_3$	0.015	0.019	47
$(CH_3S)_2P(O)OCH_3$	0.0066	0.0053	96

With the exception of phenthoate acid each of the isolated impurities, when added to purified phenthoate, caused significant potentiation. Partial data are presented in Table 3. While considerable variation in the potency of potentiating ability is evident, there is little doubt potentiation occurs. The most active potentiator was O,S,S-trimethyl phosphorodithioate where as little as 0.0025 percent caused almost a doubling in the toxicity of purified phenthoate to the rat (from 3100 to 1700 mg/kg).

A better appreciation for the potentiating ability of the different impurities may be obtained from Table 4 in which values for the amount of each impurity which causes a two-fold increase in the toxicity of phenthoate are presented. O,S,S-trimethyl phosphorodithioate and O,O,S-trimethyl phosphorothioate clearly show the highest potential activity and are approximately 50- to 100-fold more effective than S-Me phenthoate or O,O,S-trimethyl phosphorodithioate.

Table 3: Potentiating Action of Impurities on Phenthoate Toxicity to the Rat

Compound	% Compound Added to Phenthoate	Rat Oral LD_{50} (mg/kg)
phenthoate oxon	0	3100 (control)
	1	1300
S-Me phenthoate	0.1	2200
	0.5	730
$(CH_3O)_2P(S)SCH_3$	0.4	1170
	1.6	650
$(CH_3S)_2P(O)OCH_3$	0.0025	1700
	0.005	900
	0.01	600
	0.1	360
$(CH_3O)_2P(O)SCH_3$	0.0025	1920
	0.01	1240
	0.1	620

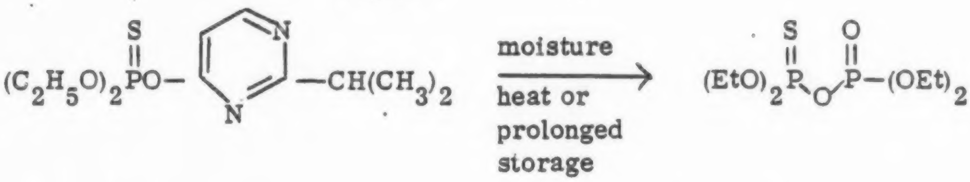
Table 4: Percent Impurity Required to Produce a Two-Fold Increase in the Toxicity of Purified Phenthoate to the Rat

Impurity	Percent Added
$(CH_3S)_2P(O)OCH_3$	0.003
$(CH_3O)_2P(O)SCH_3$	0.0055
$(CH_3O)_2P(S)SCH_3$	0.25
S-Me phenthoate	0.2
phenthoate oxon	0.75

These results clearly demonstrate that trace amounts of simple trimethyl phosphorothioates and dithioates have a profound effect on the mammalian toxicity of phenthoate and related esters. However, they evidently have little effect on insecticidal activity. Of some concern is the observation that some of these impurities are themselves somewhat toxic to mammals. This, coupled with their capacity to potentiate the toxicity of such safe organophosphorus insecticides as phenthoate and malathion, points out the need to examine other organophosphorus esters for impurities and assess the effect of these impurities on mammalian systems.

The preceding discussion was concerned primarily with the effect of impurities present in technical materials which are probably introduced during the manufacturing process. In addition to this, there is the possibility that additional impurities may be generated upon storage of technical materials, particularly when subjected to elevated temperatures. A good example of this is the decomposition of Diazinon, another "safe" insecticide, in the presence of trace amounts of water to monothio-TEPP.

Figure 2



This reaction, which occurs after prolonged standing at room temperature or much more rapidly at higher temperature, was responsible for the variation in Diazinon toxicity to mammals during the early days of its development as a useful insecticide. The problem has been solved by improved formulations. There are numerous other examples where an organophosphorus insecticide is converted upon standing to highly toxic materials, e.g., the conversion of methyl demeton to a sulfonium salt of extremely high mammalian toxicity and the conversion of dimethoate by transesterification with cellsolves to give toxic products.

QUESTION: I was wondering if any work has been done in this or other systems on the pesticide properties of some of these other compounds?

DR. ZWEIG: Yes, very good question. Now I have the answer to that one. They were found not to be insecticidal, and they were not potentiators of the insecticidal activity either. This is very interesting. They are potentiators of the mammalian system, although the mechanism of the potentiation is really not known.

QUESTION: To happen in solutions, all of them should be looked at for these trace impurities.

DR. ZWEIG: Yes. Of course there is no contract, but we have a list of pesticides that we wish the contractor to study, and these compounds are all included. This is correct; what Dr. Ross is saying is true. Several years ago there was found a malathion product, a technical product, and it was analyzed and it was found by analysis to contain TEPP. I'm just wondering if it didn't go through exactly the same mechanism.

COMMENT: It would follow the same mechanism.

DR. ZWEIG: TEPP, to the best of our knowledge, was not used in the formulating plant. It was not cross contamination and it certainly could have fallen right into the same type of reaction.

MR. PROUTY: My question to you and also perhaps to Dr. Warner is, are any of these impurities produced by side reactions, are they likely to hang around in the environment, or can we regard them as easily biodegradable or easily detoxified and excreted by the animal?

DR. ZWEIG: My answer would be, but I'd like Dr. Warner's answer also, that these are quite water-soluble, and I do not think there's a problem. They are very easily biodegradable and I don't think they will cause any problems in accumulation. This would be my first guess. Dr. Warner, do you have any idea?

DR. WARNER: I would agree with that, since these are phosphate esters like the pesticide, they should have a similar type of degradation.

DR. ZWEIG: I really don't think they're problems. They are more problems of the mammalian toxicity of the technical material. Very good point you're making. Let me put it this way since you're from the Fish and Wildlife Service, is that correct? I think someone ought to really study the potentiation system in fish. You may find that the same potentiation effect of these simple triachial esters on fish, the same as in the warm-blooded mammalian system. That I don't think has been studied at all, and I think this is worthwhile studying. But as far as accumulation, that I would not worry about. I think they are readily eliminated.

RESEARCH PROGRAM OF THE CHEMISTRY BRANCH

Dr. Edward O. Oswald*

The last subject on our schedule prior to adjourning for lunch is a discussion of the general program of the chemistry branch at RTP. I'm going to make this very brief, but at the same time try to give you some feel for our overall program and how it fits into this particular Substitute Chemical Program. Within this general overview, I will not go into great detail and data. If you'd like some of this, I can discuss it at a later date.

The Pesticides and Toxic Substances Effects Laboratory (PTSEL) chemistry branch which is now a part of the Health Effects Research Laboratory at the Research Triangle Park is divided into four sections: the quality assurance, methods development, chemical characterization, and special projects sections. During our presentations you have had a chance to meet Dr. Bob Lewis and Dr. Bob Moseman who are section chiefs within the branch. From the standpoint of the quality assurance programs, this particular responsibility responds to at least three areas: inter and intra quality assurance programs as applied to the community studies laboratories of OPP, to the regions, and to other state, local, and Federal agencies as a need or assistance in this particular area of quality assurance is applied to monitoring.

Within the quality assurance program, the inter and intra Q. C. program has been brought about by submission of standard reference materials with a follow-up on evaluation and analysis of the data generated from SRMs, or in this case, standard reference materials of various biological matrices, varying from simple complexity of compounds and solvents to those including biological matrices such as adipose or blood. In addition to the inter and intra Q. C. programs, the quality assurance section evaluates and standardizes methodology as applied to the Agency regional needs. They also maintain a centralized electronics repair and calibration facility which was originated for the community studies programs and now has been extended into the regions.

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*Health Effects Research Laboratory, Research Triangle Park, North Carolina

The quality assurance program also includes the task of maintaining a working repository, presently of some 500 compounds, through the assistance of the manufacturers. We ship analytical standards to any requesting organization at a present rate of 600 to 1000 samples per month, with approximately 550 or more laboratories throughout the world requesting information.

One of the efforts within the Q. C. program which has a direct bearing on this program but is not funded or associated immediately with the substitute chemicals is a program which we were involved with on evaluation of methodology for pesticides in water. This particular effort arose because very little could be said about methodology as it is stated in the Federal Register. We began this effort to see what could be accomplished. As this progressed through the stages of determining whether a methodology is capable of multiclass, multiresidue analysis, we found that the existing methodology could not handle a wide span of OP, carbamates, and other substituted pesticidal compounds.

An effort is now under way within the branch to upgrade methodology in water. Of the 75 or more compounds, I'll list only a few from the Substitute Chemical Program. These include atrazine, simazine, captan, methoxychlor, PCNB, azinphos-methyl, diazinon, disulfoton, parathion, paraoxon, ethyl, and methyl, including in this case carbamates such as carbofuran and carbaryl. Within this particular process, which started out to be an evaluation of methodology, we found that the methodology as it exists is not sufficient. Therefore, we have now reversed the process back to development of methodology and then reevaluation on the collaborative basis.

For those of you who are not familiar with the process, the methodology is normally developed through either contractural or internal processes; then it is put out on collaborative evaluation to numerous laboratories. Once something can be said about this methodology, it is applied as needed to various monitoring programs.

We can divide other efforts within the branch into several areas, one of which concerns detector systems, and we've heard some of the extramural efforts on this earlier. From the standpoint of intramural efforts, we have the complementary of mass spectrometry programs. I'll touch very briefly only on one of these, in this case a comparative effort for organometallics, a class of compounds which is very difficult to handle by any existing analytical methodology. From this standpoint, the idea was first developed to see if field ionization, or field absorption, coupled with non-flame atomic absorption methodology, could give a meaningful result. This is applied to such compounds as in this case the arsenicals, cacodylic acid, eventually shooting for an *in situ* analysis.

From the standpoint of ionized species, cacodylic acids and derivatives which are very difficult to isolate and/or the compounds which are chemically modified during these processes, we wanted to study the feasibility of using field absorption which does not, in this case, require a volatilization. If the feasibility were possible, this technique would be applied to quantitation.

At this time we can say that field absorption and field ionization are applicable to both organometallics and organic trace analysis. We have looked further into feasibility of gas chromatography interface with field ionization, which is an extension of other techniques you've heard about earlier and I won't go into that.

Other detector systems within the branch program include those of applying the Hall detector to nitrogen-containing pesticides, both the carbamates, nitro-containing compounds, trazines, etc., optimizing conditions in numerous biological substrates. I think you gather from the presentations that you have heard that we have research and extramural efforts in water, soils, and heavily weighted in this case in health effects-oriented substrates including human tissues, fluids, and excreter.

One of the program areas which involves a large amount of our time is developing methodology for determination of index of exposure to various types, classes, and individual compounds of both pesticidal and nonpesticidal nature. From the standpoint of the organophosphate-containing compounds, the methodology which was earlier reported by Dr. Shafik for determining alkyl phosphate metabolites in urine has been upgraded and has been applied. Numerous modifications have now been made.

One of these, which is going to be reported at the ACS meeting, was developed in order to overcome some of the problems associated with isolation of these highly polar metabolites from aqueous systems. This encompasses using an ion exchange resin to first of all extract the metabolites. Derivatization is then done on the ion exchanged resin and the materials are then chromatographed by the normal sequence of events.

Other efforts concerning the biodegradable pesticides include those of looking at the metabolites, either the substituted phenols, the amines, or the anilines. Furthermore we investigated workable derivatization techniques applied to these polar metabolites.

In addition, we have another area of research which has been directly associated with the substitute or alternative chemicals program. That area is evaluation of chromatographic techniques involved in multiresidue procedures, and you have heard some of the extramural efforts on this earlier. This particular effort involves looking at the feasibility and actual flexibility of chemical bondage GC phases for analysis of intact pesticidal compounds. This involves preparation of chemically bonded phases such as those of carbowax 20M, bonded chemically to numerous types of phases such as from the chromosorbs P, G and W, etc., and evaluating these chemically bonded phases for their chromatographic separation capabilities with such compounds as those of phosphamidon, diazinon, atrazine, simazine, disulfoton, Dursban, methyl parathion, and others, including trifluralin.

In this particular effort the chemical bondage GC phases have resolving capabilities much greater than those of the normal pack column characteristics. Extension of this, which is planned, is for applying this working GC column system to the other detectors I've previously mentioned.

Other areas within the branch programs include those of cleanup methodology, upgrading, and modification. This is always a problem as you begin to consider the multicomponent systems and biological substrates. To us a biological substrate could be anything from human adipose tissue to urine, feces, water, or air. As we all know, there are very few methods that apply to all of these. Each one has its own specific characteristics. I have already mentioned the sequence of events that takes place concerning methods development, as we see it, within our branch. Normally the method is developed and then collaboratively evaluated prior to use for monitoring purposes.

I'd like to give you one last insight into an application which happened to be a compound that by chance was included in the Substitute Chemical Program. It's a very real portion of our program, and this is furnishing assistance from time to time to Federal, state and local concerns in cases of suspected poisoning. For instance, we had received requests for technical assistance from a group that had a suspected poisoning case, OP in this instance. The patient was overly intoxicated and had consumed a material which was suspected of being an organophosphorus-containing pesticide. In addition, by unknown means, the formulation was contaminated with turpentine and a number of other substances. Through the assistance of a number of the techniques which you've heard about earlier today, namely GC, GCMS, cleanup procedures, the formulation was first concluded to contain one of two compounds, Azodrin or Bidrin. In this case chromatographic separation techniques would not resolve this, but by mass spectrometry, it was very easily discernible that this particular formulation did contain Bidrin. By translating this one step further to the analysis of the patient's urine, it was found that this particular patient was excreting approximately five parts per million of the dimethyl phosphate. Therefore by correlating this information in an everyday working fashion, this gives you the type of problems we are concerned with. At the same time, there is always a need for increasing the specificity and sensitivity of methodology as it applies to multicompound, multisubstrate systems.

DR. MOYE: You mentioned the work of Dr. Shafik along the lines of index of exposure, and he has developed, I think, some very significant methodology for the cholinesterase inhibitors, organophosphates. Does your branch plan to extend this to other classes of cholinesterase inhibitors, that is, to develop methodology for index of exposure? I'm specifically thinking of the carbamates.

DR. OSWALD: Yes. We have within our programs at this time areas which include both the intact and the transformation products of the carbamates.

One of the problems which we have found, as I think you've already experienced, is stability of compounds, both from a derivatization standpoint and from an analytical standpoint.

In the case of the perfluorobutyl or the PNB derivatives, depending upon the compound, we do find problems. In relationship to the index of exposure as monitored by urinary metabolites for OP compounds, one of our biggest problems is not necessarily due to instrumentation drawbacks. It is due mainly to reagents which have nothing to do with what's in the urine, but is really what we use to fabricate the derivatives.

DR. ROSS: I thought the analysis of pesticides and water was a primary responsibility of Cincinnati. What is your interfacing or coordination with their work? I'm talking about Lichtenberg's manual notes on organophosphates, chlorinated phenoxides, and carbamates.

DR. OSWALD: We found, like everyone else, that no one can do everything. In this particular effort we were requested to at least attempt to say something about methodology; this is on the Federal Register at this time.

The type of involvement, whether it's water, air, soils, or tissues, in relationship to our effort, I would have to put on a substrate basis. Our heaviest involvements are those in health effects and human exposure systems, and next to that would be air on down to soils with water last.

Ours was really a supplemental effort to that of Cincinnati.

DR. ROSS: In other words, you're overlapping in methodology because it is the responsibility of Cincinnati and not RTP to develop methodology for pesticides and water.

DR. OSWALD: It was supplemental.

DR. ROSS: With reference to substitute chemical cleanup methods, you were investigating the feasibility of this some time ago. What progress, if any, has been made on that?

DR. OSWALD: In relationship to cleanup methodology, one of the areas which I didn't touch on in-depth -- this includes GPC as applied to multiclass, multi-performance systems -- we can say at this time with existing methodology that modifications are required.

DR. ROSS: GPC?

DR. OSWALD: Gel permeation chromatography. You cannot take most of the reported systems which were initially developed for adipose and apply them to systems with different interfering problems. We have heard about some of these from Dr. Hill and others. We're hoping to resolve some of these at a later date -- at least as it stands right now, taking methodology for GPC for soils below a tenth of a part per million. We do have to come up with additional cleanup methodology.

DR. ROSS: Most of your methodology seems to be oriented toward soil chemistry rather than adipose tissue. Is it weighed differently? Are the priorities greater now for soil than for tissue?

DR. OSWALD: If you look at our responsibility for methodology, for monitoring, especially from a regional standpoint and as we support the Office of Pesticide Programs community studies labs, you'll see that they've been diverse activities. In the regional situation, the only common involvement that one region may share with another is probably that in water. We have found, through the clarity of evaluations and the SRMs, some regions do not have any involvement in human-associated fluids. One of the first reasons for being involved with water was that all the regions had a common need for methodology in water. From the standpoint of the community studies laboratories, their programs have been oriented toward that of human tissues, fluids, air, and water. The varying amount of involvement is the reason you see a combination of all of these substrates in our involvements. We do support all of these programs.

DR. WARNER: A number of years ago, the old chemical corps spent quite a bit of effort on developing colorimetric systems and field kits for determining the presence of organophosphate CW agents. Have these systems been looked at very thoroughly for the pesticide problem?

DR. ZWEIG: Yes, they have. It turns out that many of the methods were based on cholinesterase inhibition. We wanted to get away from stabilized enzymes so we were not happy.

Secondly, most of the apparatuses developed by the Army at that time were extremely costly, very cumbersome, and very complicated, and when we checked out the sensitivity they were really not suitable for the sensitivity that we were looking at. They weren't looking at tremendously large contaminated areas. We were talking about nanogram quantities, and they were talking about milligrams per liter.

We spent quite a bit of time at Edgewood talking to people like Cramer and Gamson. None of their methods, with the exception of the method that Stanford Research picked up, which was also developed on contract for the Army, seemed to be suitable.

We were looking for simpler methods and, as I mentioned before, this effort on our part was for a much simpler method.

There was a method that was developed, I think by Cornell Lab, also on the contract with the chemical corps, that is a so-called "ticket." We are still looking at this, and possibly this ticket might be funded by Research Triangle Park.

Nobody can tell us what's behind the ticket. It's a plastic tag and the idea is similar to a dosimeter that you carry on your lapel. According to the inventors, who won't tell us what's inside the ticket, it turns from a green to a red depending upon how much phosphate is present, but it's very nonspecific. I think what they're really depending on is the solvent carrier of the pesticide, not the pesticide itself.

When we thought about the system, we liked the idea of a dosimeter that every person carries with him, and if he is in danger, it will turn a different color.

The ticket was investigated by the Office of Pesticide Programs and the Operations Division under a project called "Operations Safeguard" several years ago when they went to parathion. It has not been found to be too useful, but it is still being investigated.

To answer your question, yes, we have looked at all the things that are declassified, and we decided that with the exception of the one system that Stanford picked up, none of the others seem to be suitable for our particular purpose.

DR. LEWIS: I just wanted to point out that there are two aspects to the hazard involved with reentering freshly sprayed fields. One is air route exposure of a person to the contaminated air in the area. But, I think that is secondary to exposure from dislodgeable residues. There has not been a satisfactory method developed for determining dislodgeable residues. All you can do is take foliage samples and wash them off with water or various solvents. We are currently only looking at the air route of exposure, even though it may be the less important.

COMMENT: We do have a very small effort that we did not mention here because it was not funded by the Substitute Chemical Program. It's exactly what you're just stating, and that's a quickie method for determination of dislodgeable residue on fruit and leaves. The thing looks like a nutcracker. Basically what you do is put into this nutcracker device a leaf and a filter paper strip and as you press the handle together, you slowly rotate the little wheel which wedges the leaf against the paper and actually rubs off 100 percent of the dislodgeable residue and everything else.

It waxes the chlorophyll and the pigment onto the paper. Then this paper is developed through a very simple solvent system and in a tiny test tube -- a 10-minute development just like a paper chromatogram -- and the paper has already been pretreated with DCQ coloring agent and you get a color. The color that is developed is a band; in a way it's semi-quantitative, directly proportional to the concentration.

This is under development now. This was a very small contract, but it seems to be a very simple field test for farm workers or somebody to actually measure the dislodgeable residue. This has been written up as a manuscript and will be published in the Bulletin of Toxicology probably in the next couple of months.

QUESTION: How's the sensitivity experiment?

COMMENT: Within the range that we're looking at, Riverside has a contract to study the overall concentration of pesticides in orange groves. Part of it may be a correlation between the cholinesterase levels and levels in the urine.

It's a much more comprehensive program. This little bit where they developed this method of dislodgeable residue is within the actual range of what is found out in the field after one day, two days, and so on. It's quite sensitive and, as I said, it's semi-quantitative. There are some problems. The coloring agent's not too stable.

You have to measure your color. You have to heat the color. There are certain things that are not totally worked out, but they have shown the quantitative relationship between the amount of residue and the concentration and the color.

SPECIAL INTEREST TOPICS AND GENERAL DISCUSSION

DR. OSWALD: During the presentations given yesterday and today we have heard discussion on various topics including GC detector systems such as the Hall; multiresidue methodology; the multiplicity of problems and complexities involved in identification of impurities; problems associated with reentry; exquisite instrumentation; newly planned efforts, including those of mass spectrometry and automated cleanup methodology; continuation of multiresidue methodology; research related to pesticides in air; means and detections of pesticides in the field, again related to reentry problems; and potentiation of pesticide as related to contaminants or potential contaminants.

We have discussed these areas to attempt to further scientific knowledge and to answer the questions from the chemical standpoint to what is there, how much is there, and what does it mean.

Before we go into other topics which the audience may have interest in discussing, I'd like for you to think back through the presentations and if there are any comments concerning the plan of the program, the design of the particular program, its emphasis, specific information which may be pertinent to various compounds or classes, now is the time you can discuss these. Feel free to make any comments at this time.

DR. J. ROSS: One question I have concerns the list of compounds that are used for the work in this program. These compounds are not among the compounds that are being considered for substitute compounds, and there's a wide-ranging list. What is the basis of selection for these?

DR. OSWALD: As was explained in the general session -- and I think you'll probably hear some more about this -- there has been a coordinated effort to determine on a use basis what compounds may serve as alternates or substitutes

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for the following persistent chlorinatives: DDT, aldrin, dieldrin, chlordane, heptachlor, substitutes for 2,4,5-T, the ethylene bisthiocarbamates, and certain fungicides. The matrix which has been prepared on these is associated with that of a crop/use orientation.

DR. ZWEIG: There was an article in C and E News -- June 9 -- which listed all the compounds that are under consideration right now, which are potential substitutes for DDT, aldrin, dieldrin, chlordane, heptachlor, 2,4,5-T, and EBDC. This is pretty much the list that we go by. The list will be expanding as we consider, in all probability, chlorinated pesticides for Akton, but you're totally up to date as of yesterday. I think you have the total list of pesticides that are now being studied for litigation, already have been cancelled or suspended.

As Dick Back pointed out yesterday during the opening session, a compound like carbaryl was left out as a substitute for DDT. This is not an all-inclusive list; this was the best possible list that we drew up.

Carbaryl --and this was not explained yesterday-- was not a substitute chemical as far as our internal bookkeeping was concerned because we had looked at it from another point of view in an internal review.

I should explain to you that the Agency is looking at substitute chemicals, and this is one thing, but we have other compounds for internal review as, for example, chlordane/heptachlor. These are the compounds that are being thought about for litigation.

Carbaryl is a compound that we had inherited from many years back when there was some question about its safety, and it had just gone into the mill of the internal review. Actually we have looked at it and yet, in a way, it should have really been one of the prime candidates in the Substitute Chemical Program. In most of the multiresidue methods carbaryl is always chosen, and in the air pollution studies and in all the other work that was reported here carbaryl was always shown as a prime compound, representing the carbamates.

I would say that this list is quite good, but it is not totally inclusive. As we are looking at other substitutes for other compounds to be considered for litigation, undoubtedly this list will expand, but there's nothing particularly significant about the choice of any of these. These compounds came out of a computer match-up, as Dr. Oswald pointed out, between the use pattern -- in other words, the site and the insect, the corn borer against corn. If this had a registered use and this would substitute for aldrin, then it would be considered as a substitute chemical.

Most of our contractors and cooperators are adhering pretty much to this list, but if they want to choose additional compounds that are more representative of their chemical methodology, we have no particular objection to it. It would expand the horizon of the methodology. Does that explain it?

I do have a question to add. We have discussed very sophisticated methods, including ESR and hyperfine labeling, mass spectrometry, electron impact, and chemical ionization. I would like to ask Dr. Hill to tell us a little bit about the simple method which is still very useful -- TLC -- and I know that he has some efforts in his lab right now. Maybe he can tell us a little about that. That's still a good method. It's easy, very cheap, very simple, and very sensitive.

DR. HILL: Unfortunately, the TLC plates did not survive the local humidity for two days, so I'm not going to show them. We've been very fortunate in having Professor Joe Sherma attached to our lab this summer as a faculty appointment. We brought him in on a "do anything you please" basis, as long as it's in TLC.

After he came onboard, we kicked around a few ideas, and one thing that's always been in the back of our minds for years is a quantitative TLC procedure using optical scanning devices which is pretty well along for many compounds, using conventional color development, but we're talking about samples down in the range of micrograms, maybe one tenth if you're reasonably good, a half a microgram if not.

We all know that cholinesterase inhibition techniques for TLC are extremely sensitive, but the plates are usually messy. You wind up with a cholinesterase inhibition white spot on a blue or some other color background, where you've inhibited some reaction. Joe and Mr. Getz decided to see if they couldn't get a decent looking, scannable TLC plate, using the cholinesterase inhibition approach, and in the first few weeks, they met with success beyond their expectations.

The first approach was very conventional, but they very quickly realized it (the white spot on a black or blue colored background) was not terribly useful, even if you managed to clean up the background so it is a uniform color and not streaked or mottled or spotted. The white spots are simply areas -- the concentration is a function of the area. There is no optical density to deal with here. It's either white or not at all, and so the idea was to get a color reversal plate, which they have managed to do now. We get blue spots on a white background, and a very white background at that.

This spot is proportional to concentration with the usual plateau in the higher concentrations which is a characteristic of saturation. This was done by using horse serum, and the acetothiocholine is hydrolyzed by the cholinesterase enzyme to form thiocholine and acetic acid. The thiocholine has the ability to reduce the blue redox dye (2,6-dichloroindophenol) to a colorless or yellow form.

You dip your plate into the dye and get a solid blue plate. In about 30 seconds it just simply bleaches out white, except where the pesticides are, which stays blue. The area of inhibition is proportional to the amount of pesticide present, and we're down in the nanogram range.

I had hoped to be able to show you some of the actual plates. As I said, we made some up Tuesday morning before coming down here and kept them in the refrigerator all day with a cover glass, but they didn't survive.

QUESTION: Did they survive long enough for scanning?

DR. HILL: Yes, in the lab there's no problem. You can keep them overnight in a refrigerator and they're quite good. It's just that, after coming down here in a hot car, it was too much for them. What actually happened is we got another reversal back to white. These were originally bright blue spots on a white background, but they faded out. This was down at 32 nanograms--the one we were hoping would survive and didn't -- you see the dye is back again. This was a white plate, again with blue spots, and this is 2, 4, 8, 32 nanograms of phorate. Then, just for the fun of it, they put on carbofuran and the 3-hydroxycarbofuran which also gave very good spots. They didn't try the carbonate until this plate.

This plate was one week old before I brought it down. It had been in the refrigerator that long, but it immediately faded out after one day at room temperature. They are about to wind this up, and I think they're going to get something interesting published on this very shortly.

QUESTION: Are these commercially available plates?

DR. HILL: Right. They're silica gel plates. "Quantagram" is the one plate they're concentrating on for the moment. They were using Merck plates before that. When it comes to the dip technique, which is critical to this, the stability of the plate coating is critical.

The Merck plates were very hard to the touch -- physically, you could scratch off the Quantagram plate before the Merck plate. When you get the thing wet (apparently the binders get involved) in the dip tank, the Merck plates slough off and the other ones hang in there.

QUESTION: How about the Eastman chromato-sheets?

DR. HILL: They have not tried any of those. They're not stiff enough to be that reliable for scanning. That's one of the problems.

QUESTION: Is it by reflecting?

DR. HILL: This is by reflecting. Yes, I didn't want to go into that because of my personal involvement.

QUESTION: Is it area or optical density that determines the intensity of the spot or response?

DR. HILL: It's both. The response of the instrument is designed and based on reading both the area and the optical density simultaneously. The total signal that you see -- the integrated optical signal -- is a result of both of those factors. Either one alone will not give you a linear curve, but combined they will. Of course you have to use area -- you cannot use peak height -- because that may not have any relationship to concentration because of the shape of the spot or something. We're using a Kontes scanner which they lent us.

QUESTION: Is that a scanner that scans across or can you position it in one phase?

DR. HILL: Essentially you position your plate, and then the scanning head is positioned by eye over the row of spots here, and scanned in this direction. The plate moves under the head with a cover glass on it, and the scanning head rests on the cover glass, which is part of the patent, and you can scan six rows of spots that way.

To show you how well this really works, we've just finished a two-year study of carbofuran in soil and alfalfa at Beltsville, with John Neal, an entomologist, in which we did all of our analyses by TLC. This included acid hydrolysis as a first step to break up the conjugates and all that.

QUESTION: Can you give any idea of the linearity and sensitivity?

DR. HILL: The linearity for any TLC plate is always the same. We will always see something about like this, and it doesn't really matter much what your units are here. You'll have about a decade or a decade and a half of linearity before it flattens out, and this may very well be nanograms or it may be micrograms, depending on what kind of plate you're using.

This type of thing is limited to about a decade or a decade and a half. We can usually push it to a decade and a half with the cholinesterases -- I've seen some of the plates that have been running anywhere from two nanograms or less up to 32 before they begin to flatten out.

QUESTION: You were not using plant extracts?

DR. HILL: These are standards, right.

QUESTION: Do you have to activate the organophosphates with bromine?

DR. HILL: They were trying that. I don't think they're doing that now.

QUESTION: Is the spotting technique for this scanner critical?

DR. HILL: Yes, we're using the Kontes spotting device which was developed in our lab by Mr. Getz and Dr. Beroza. If you'll consider for a minute what you're doing in pesticide residue analysis -- you fill up these little glass tubes with the syringe needles on the end and then put them in this device, and it drains out by gravity flow onto the TLC plate. It's made in such a way that we have an air manifold underneath through which you can bring in compressed dry air or nitrogen, and this evaporates your solvent at a fixed rate and controls the spot.

It allows you to put on two or three milliliters of sample containing your pesticide after you've concentrated it down. You can put two or three milliliters in these tubes and put it all on the plate, whereas if you do any other type of spotting, usually manual, you have to concentrate down to a very small volume, and then transfer it with capillary pipettes.

COMMENT: Two or three milliliters must take a little while.

DR. HILL: No. You can just put it on and go have a cup of coffee. It can be up to a half an hour or so, but it controls not only the size and the quantity of the material you transfer, but it also has other benefits.

Of course, we're spending a lot of time, you might say, in an educational effort for quantitative TLC in which we try to tell everybody who will listen that the instrumentation is not the limiting factor, and we're not talking about the Kontes equipment any more than we are anybody else's. The machinery has been available to give you plus or minus one percent. It's just a matter of learning the operator skill and you would not expect any less of a man who was using a gas chromatograph. It's just that TLC grew up in an era of qualitative, sloppy technique, and it's just an educational thing.

The quality of the commercial plates has come along enormously in the last five to eight years. Initially, we only used homemade plates; now we trust the ones that you buy. You still get an edge effect. The Kontes spotter forces you to work in toward the center of the plate, and you can't crowd it. You are limited because of the optical design to six rows. Six rows is just about optimum for one analysis per plate. You can have three concentrations of standard, your unknown sample, and a control extract, all on one plate for background.

Incidentally, our motivation behind all of this is not to undermine GLC, but TLC is still very much the method of choice in many countries. Some countries are constantly complaining that the United States is so preoccupied with their expensive equipment. This is really an approach to get at the basic economics of it, and even the cost of the instrumentation is kept in mind because there are many countries that are now getting into pesticide problems, and we're going to have something.

DR. ZWEIG: I would like to raise another topic that has been either forgotten or conveniently ignored. We've all become very much interested, as you will hear later on this aftenoon in a talk by Dr. Phillips, in insect hormones and pheromones, and this is a real problem. So far we chemists -- I still include myself among the chemists -- have been very fortunate that we're working with compounds that have a marker -- either they have a nice nitrogen, a sulphur, phosphorus, a chlorine or something. What do we do with things that just have CH and O? I just would like to bring this up as a discussion point. Have any of you had experience with the analysis of, let's say, the commercial insect hormone (that would be Altosid) -- that's the only one I can think of at this moment -- and the Thompson-Hayward compound, but that's not in the same class, that's in the pheromones like Gyplure and the insect hormones of the Altosid type? What are we going to do about these? These may become more prevalent in the future, and what are we going to do as residue chemists? How do these compounds fit into the multiresidue scheme?

DR. HILL: Yes, I can address two areas of this. First of all, it's not Gyplure, but disparlure that is undergoing extensive field testing in Pennsylvania and other places. In one of the projects in Beltsville, they were concerned with an analytical method for disparlure to use this fall.

Jack Plimmer's lab is in charge of this. Mr. Holden from our lab is on this project, and the only thing he or I were ever able to come up with was a derivatization based on the epoxide functional group. Essentially, without going through the whole molecule, they're simply looking at what to do with this. They are going to open this up and hang something on there, something that will be electron-capturing. The decision of what to do with this has not yet been made, but this is the approach. The other compound, which is not exactly in the category of the things you're talking about, but since you mentioned Altosid, the developmental compound TH-6040, which is not a synthetic juvenile hormone, but a substituted urea, which, if you look at it, you'd swear was a herbicide, but it affects cuticle formation in insects, and it has been considered as a possible larvicide in animal feeding. We've been looking into that and the method turns out to be quite simple. Liquid chromatography with U.V. detector measures the absorption of these aromatic things even though fluorine and chlorine are in there; you can get by with virtually no cleanup.

DR. ZWEIG: That's why I don't consider the TH compound the same thing.

DR. HILL: Everyone of these -- a generalization, I'm getting in trouble -- but virtually all of these pheromones must have something this way from which you can get a derivative. They're not completely inert hydrocarbons. They will either have a double bond or something like this.

COMMENT: We have the same concern in terms of residue and tolerance setting, and fate and movement in the environment.

DR. HILL: Yes, I realize you're obliged to do that in regulatory agencies. Our interest in an analytical method for this -- and when I say "our" I mean Jack Plimmers' interest -- is for scientific purposes only, as a screening and survey device to find out where this compound is. He's not interested in developing methods because he thinks there's a residue or a residue hazard, either one. It's a purely scientific tool.

QUESTION: I haven't done any work on these hormones, but you have similar problems when you start looking at drugs, like marijuana or tetrahydrocannabinal-type compounds. There's no handle, and you have sensitivity and selectivity problems, and the derivative route has been used. It has problems in that you derivatize other things in blood and urine, and in that case something like mass spectrometry is necessary. You end up using GC mass spec to get whatever quantitation you can. It's expensive, but this may be where you'll end up too.

DR. MOYE: I hate to predict things, but it seems to me that we may be overlooking the fantastic advances in various types of column chromatography, namely high-speed liquid chromatography, in terms of the separatory power of the columns themselves.

I suspect that molecules of this sort, that don't have any handles that we can get hold of, we may find by a combination of separatory techniques — I'm thinking in terms of a molecular-sized separation of a very narrow band width, where we can pick out a fraction of molecular size, say from 200 to 210 molecular weights, and be very sure that we have that, and then pump this fraction into a second column that will perform a separation in terms of partitioning or absorption -- we might be able to get away with a very nonspecific detector such as a UV or a flame ionization detector.

DR. ZWEIG: How are you going to distinguish between this and the natural constituent, if you're into natural constitutents? What is your handle? What type of UV absorbence do you have that's specific? Frankly, if I may prophesize and look into the future, I would say the only way to go about it is by a specific derivatization, where you put the handle on. You need a handle. Otherwise, you just have a long-chain fatty alcohol, or long-chain fatty aldehyde type compound. Where do you know it's going to come off the column with a nonspecific UV detector?

DR. MOYE: The idea is to have a standard that you are convinced of what it is.

DR. ZWEIG: With a tracer?

DR. MOYE: Not necessarily with a tracer, but with large amounts of a material. If you're talking about nanogram quantities, you may be hard put detector-wise, but if you can get large amounts, you can work on a macro scale, and work out the separations.

Once again, I think we may in many ways be overlooking the extreme selectivity of some of these newer liquid chromatographic columns that are coming along. By parlaying, if you will, several separatory modes -- molecular size, absorption, reverse phase, or partitioning -- the degree of selectivity may be somewhat surprising in that we can pick out a material such as these compounds present in fairly small amounts, along with large quantities of not too different structure. A difference has to be there, and that's what you're banking on.

DR. MOSEMAN: To reinforce what Anson had said, we were able to separate acetonitrilic acid and citric acids extracted from water on a surface-bonded gas chromatographic column. This separation could not be done on any commercially prepared, standard GC column packing, of which we knew. I think there's promise in the resolving power of certain columns, particularly high-speed liquid chromatography, and it can be done.

QUESTION: Yes, but as the conference has pointed out, we're hung up on specific detectors. At least, that's the take-home lesson I'm going to go away with. Here we don't have a specific detector. We're going back to the old flame ionization or we're going back to UV detector -- nonspecific detectors. I haven't had any experience with these compounds, but I do know that -- the man isn't here from Zoecon -- when they came in to show the residue picture of this, they had to go through all sorts of very difficult separations, and then in the end, they had to use a flame ionization detector. It was very difficult for them to convince us that the signal that they got was really due to Altosid or one of its metabolites.

I think it's a real challenge to the residue chemist to develop separation and and detection techniques.

COMMENT: If you don't have a way to get at an element-specific detector or to use an element-specific detector, I'm simply saying that there is the possibility of resolving components in a mixture, and looking at them with a relatively nonspecific detector system. Granted it's not the best way to do it, but if that's the only thing you've got —

QUESTION: What about using the Beroza technique of sticking the insect? You have a cage of the end of your gas chromatograph and you see how the insect behaves. As a matter of fact, it has been used as a chemoreceptor for anti-feeding compounds. I'm just throwing this out. That's where your specificity is, in their biological response and not their chemical response. Maybe we're taking a step backward when we talk about this, but let's not forget some of these biological methods.

COMMENT: That's specific detector. I'm not at all convinced that specificity is as great as we would hope either. I think some of the work of Rolloff with the electroantennagrams show that these moth antenna can respond to a wide variety of closely related compounds that are not necessarily pheromones. You could be slightly fooled by it, I think. I'm just speculating out loud.

One other comment which is more political than scientific is that there seems to be a neurotic preoccupation in EPA with sensitivity, from my point of view, rather than selectivity. In other words, when I promote within my own Agency, the interest in something like this LC flame ionization, I'm very careful to not spell out the possible advantages it might have in sensitivity, even though it's there.

I'm using the argument that the selectivity is improved. I don't know if pure sensitivity by itself is necessarily a highly desirable thing when you're talking about something like these compounds, and that's why we were satisfied to use UV detector with an LC for TH 6040 even though we were looking for it in milk among other things.

Until we get some feedback that there is a real toxicological concern about the compound, the sensitivities are of secondary importance, not out of the question, not out of the picture, but of secondary importance.

COMMENT: I don't think sensitivity is the hang up of the Agency. I think sensitivity is something that we as analytical chemists have been totally guilty of. I once wrote a chapter called "The Vanishing Zero." We are the ones who have been pushing back the zero all the way down, almost to the single molecule. Obviously, when EPA sees a method coming along and they can only detect microgram quantities, they say, "That's not good enough. Why can't you detect picograms?"

COMMENT: We're spoiled. I think that's it. It's a natural human phenomenon.

COMMENT: In other words, the analytical chemist has gotten himself into --

COMMENT: We've gotten ourselves into a bind. There's no doubt. We always say selectivity and sensitivity, the two together, ought to be considered as a total factor.

DR. JOHNSON: I was going to bring this up in general, but it does relate to this particular type of compound. Amino acid techniques are being used for precisely this kind of application for drugs and other things where you have considerable selectivity and sensitivity, and they are very compound-specific.

You're not going to have a general multiresidue type of thing. I haven't seen or heard much from EPA as far as sponsoring programs that might use this technique for some of these newer compounds.

DR. OSWALD: In relationship to the question on amino acids, we do have small efforts, at least within our division at this time. One of the efforts was directed toward, if I remember correctly, metabolites of benomyl. It was a feasibility study to see what could be done. The results were positive and as we find more and more compounds which just do not fit any one scheme -- when you talk about handling or analyzing by some way -- the best way we can do this is to consider chemical, instrumental, chromatographic, biological, and anything else. We grasp for that extra nonexisting solution. At this time, that's what we have to do .

DR. MOYE: Dr. Zweig mentioned nanogram amounts versus microgram. I think, quite frequently, people working at residue levels limit their vision to sample size, that is to say, the sample sizes that have historically been used in terms of environmental work. In most cases we have the advantage that we can dig as much dirt or pump as much water or as much air as we need, but when you get to tissues -- animal tissue and that sort of thing -- you're talking about something else.

One thing that was driven home to me was the fact that on a liquid chromatographic analytical column, you can inject, under the right conditions, as much as one milliliter of extract, as opposed to one microliter that most gas chromatographic columns can handle. This, relating back to the original sample size, gives you an extreme sensitivity.

DR. OSWALD: We have covered pheromones, juvenile hormones, and others. Are there some other topics that should be discussed?

DR. ZWEIG: I may be in a better position to bring up these points because these are things that we're facing every day, and you are the people who, hopefully, are going to give us the answers.

How are we doing with toxaphene? I heard toxaphene mentioned once this morning. I know that the people at Cincinnati have developed a method for toxaphene, and it's based on mass spectrometry, but I cannot describe it to you. It's been published, and maybe one of you read the bulletin in which it was described. I'd like to throw this out for a general discussion -- how are we doing with toxaphene?

DR. OSWALD: The methodology which Dr. Zweig was referring to for toxaphene water is basically a GC multiple ion detection method based on toxaphene components. I think most of you realize that the analysis of any multicomponent pesticide in a biological substrate, whether it's toxaphene, PCB, or whatever, is difficult. If you look at the other techniques, you're comparing peak positions on two or more conditions and hoping for the best. In that case, your specificity is just about gone down the drain. The same type of problem exists with PCBs. You would like to convert all of these to one end product and, depending upon the conditions of the sample, the number of other things, some of this is feasible.

In the case of toxaphene, there are other problems. The only thing I can address, at this time, toward a solution or at least with the type of involvement which we have in this particular category, is that we are looking for an index of exposure to this type of compound, not looking for the relatively nonpolar material, but looking more for the polar metabolite as an indication. Anyone else like to comment on this?

DR. ZWEIG: In other words, you don't think much of the Buddie method?

DR. OSWALD: I didn't say that. No, what I am saying, since you asked me to describe it, it is a GCMS mass spectrometry multiple ion detection method. With some of this specificity built in, I think it's functional. I really can't comment anymore than I have -- I haven't seen it in operation that much.

There is a general comment which I'll pass to all of you. As we begin to look at an environmental sample and ask the question, "What are trace levels?" and look at the techniques used, whether it's chromatographic GCL or whatever, the first question that always comes up when you get what you feel is an analytical result in relationship to validation is how specific is the method? This is something we've talked about these two days.

Specificity comes in a lot of camouflaged coats; some are easy because of the way the molecule's built, others you have to build in specificity -- either from the standpoint of cleanup, detectors, or other techniques.

The instance that you brought out on the pheromones, juvenile hormones, you have to build, or at least start from some point, and subfractionate, and build in specificity just by use of techniques alone, working from an unknown to something you can give by polarity differences, chemical characteristics, and finally say, "Yes, this is something which, even though the conditions are fairly exact, you still have to verify the other means."

DR. HILL: I have comments on the toxaphene question. If you're talking about toxaphene, you're talking about an American product -- Hercules. If you're talking about camphechlor, then you're talking about products that are made from Lord-knows-what-kind of pine oil to begin with, wherever the pine trees are grown. There are two questions here.

If you're talking about toxaphene, the American product, and most of the residue analytical chemists that I know--certainly those in my lab--can follow a toxaphene signal on a GC on into the noise level. They've done this in many instances of actual environmental samples, such as a massive toxaphene spraying program in the Southwest, where we were part of a group of labs looking at water and mud samples taken from reservoirs and rivers. Even though we had some oil from the pumping mechanism in this sample, we were easily able to follow the very characteristic toxaphene signal down to the parts per billion level.

The question of toxaphene or camphechlor -- camphechlor is another issue -- arises from the international interest in this compound. The toxicologists will raise the questions, "Well, they've seen the data on toxaphene, not the components, but the mixture and, internationally, as this camphechlor is being circulated and used, is it going to be identifiable? Is the analytical residue signal going to be the same or is it even relatively the same compound?"

That question needed to be answered. The problem is rounding up enough samples. I made an effort through my connections with FAO to have their field offices send in samples. We didn't get a tremendous response. We have three foreign samples and even their history is a little dubious. I just received word last week that Professor Cassda will accept these samples and do a mass spec comparative analysis of that with the American-made product -- look for and spell out any similarities or differences. This is a big step toward answering the toxicological concerns about this material more than the analytical.

There are some interesting things about toxaphene. I'm surprised it isn't on the substitute chemicals lists instead of being on the other side of the fence. It's obviously being used as a substitute for a lot of halogen compounds that are on the way out, and as Cassda says, it's biodegradable.

Because there were fish kills associated with toxaphene spray dips on cattle several years ago, I ran an internal experiment at Beltsville in which a herd of calves was simultaneously spray dipped with toxaphene, and every day and then at periodic intervals thereafter, the calves, in pairs, were waded through a one-foot deep rubber or plastic swimming pool, and the water was taken for analysis.

We were trying to find some practical way of finding out how long this stuff hangs in the hair of the cattle and can be washed off their legs when they go wading in the local creek. We were having bioassays done by another lab on the fish.

We were able to follow this toxaphene down to about 40 or 50 days, at which point it was still giving a reasonable amount of toxicity to fish. We were having a little problem with the curve, because the calves would lay down in the pool, and you'd have a big peak. They'd urinate in the pool, and you'd have a problem. It was a very in-house thing. It's never going to be published; it's a little bit too crude for that. It was just to find out how long this stuff remains when the farmer spray dips and then lets them go in the field, and they wade around in the local water supply. It stays on those legs a long time.

QUESTION: Can your chemists, when they get a total unknown source sample or water sample, identify toxaphene among all the other peaks that they find?

DR. HILL: We could take one approach. I won't say that we've actually done this, but we've done the research to back it up, and that is, toxaphene is a fairly easy compound to destroy and to get by difference, that is, if you have a signal which looks like toxaphene, you can treat with a reagent, and you can very readily remove it entirely from the chromatogram or you can bunch it up into one corner with a small number of peaks. I think some photochemistry has been done in the same vein. Under reduction toxaphene is very sensitive to reducing agents. It's not sensitive to oxidizing agents, which is one of the big virtues in environment, as far as its field treatment incorporation into soils or muds. It should be reduced fairly readily under these conditions.

DR. LEWIS: Toxaphene like PCB, except even worst by virtue of the fact that it is made up of so many compounds, is a problem from the environmental monitoring standpoint. All of these compounds are not going to stay together in the same ratios, and any method that depends on identification of toxaphene as an intact mixture of compounds is going to have problems from a quantitive as well as a qualitative analytical aspect.

QUESTION: Do you find great changes in the pattern we have just experienced here? I'm talking about field water residues as toxaphene signatures.

DR. HILL: Toxaphene disappears, but it doesn't change the pattern enough to not be recognizable.

DR. LEWIS: Again you're looking at situations where you knew what was there to begin with, and you have a little bit of built-in bias. Using a relatively non-specific detector -- and speaking of specificity, electron capture is not much better than flame ionization -- those peaks that you're attributing to toxaphene components in looking at ratios and patterns and whatever could be something entirely unrelated to toxaphene.

DR. HILL: The advantage we had -- it's also the point I'm making -- is that since we knew it was toxaphene, we were able -- right down to the point where we couldn't see it anymore -- to still compare those signature pieces, if you will, and the shape of the pattern and the ratios were not so much different for us to be convinced that there was any drastic shifting going on there.

MICROELECTROLYTIC CONDUCTIVITY DETECTOR

Jack B. Dixon*

NOT AVAILABLE

FOR

PUBLICATION

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